Plants as biotic indicators of Neogene palaeoenvironmental evolution in the Cape Floristic Region

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Vera Hoffmann, April 2012.

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ABSTRACT

Comparative biologists have refined the synthesis of molecularly dated phylogenies and ecological data into an important tool to reconstruct the evolution of species and biomes, and to unravel the history and role of abiotic determinants of diversity patterns (fire, climate, tectonism). This has been extended into the cross-disciplinary, geobiological approach of 'geoecodynamics' that exploits the spatial fidelity of locally restricted organisms to unravel the temporal and spatial evolution of landforms. This research approach is adopted here across 11 plant clades representing six prominent plant families of the Cape flora (Asteraceae, Orchidaceae, Restionaceae, Cyperaceae, Poaceae and Proteaceae) to infer (i) the relative roles of climatic changes and neotectonic uplift in shaping the CFR since the Early Miocene, and to determine (ii) whether contrasting evolutionary processes (adaptive versus non-adaptive) exhibit spatial structuring within the flora, given the complex topography of the region. Congruent patterns in reconstructed habitat endemism across 10 of the 11 groups suggest that the Cape flora originated in oligotrophic, aseasonal habitats that resemble present-day conditions of the region's predominantly quartzitic montane habitats. Molecular dates on these ancestral nodes range from 7 to 21 Ma, suggesting that these high-altitude, aseasonal habitats have provided climatic refugia allowing the persistence and gradual accumulation of species since at least the Miocene. Since then, the earliest shifts to seasonal habitats (4.68 - 13.35 Ma) somewhat pre-date convergent shifts in 10 of the 11 lineages to shale- and calcrete substrates (4.21 – 6.48). These landforms reflect exposure and induration, respectively, in response to tectonic uplift through the late Neogene. Shifts to higher diversification rates occurred in six groups since the Miocene/Pliocene boundary, but did not consistently coincide with the proposed timings of either tectonic uplift or aridification given in the literature. By contrast, the remaining five groups diversified at relatively constant rates over time, with either rapid decreases in rates since the Pleistocene or diversity-dependent rates, which may constitute signals of 'ecological saturation'. The unexpectedly low number of shale-endemics recovered in these sampled clades (5% shale- versus 51% quartzite-endemics) possibly reflects an edaphic

control that constrains the exchange of lineages across the eutrophic-oligotrophic divide within CFR. In order to fully understand the environmental and floristic history of Cape landscapes, the results of this study identify the priority of incorporating lineages with a closer affinity to eutrophic soils. Finally, the study highlights the utility of plants as biotic indicators, which can complement and extend established methods in geochronology and geomorphology to quantify aspects of historical geomorphological change in landscapes at a spatial and temporal scale currently unattainable by traditional geomorphological methods.

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CHAPTER 1: INTRODUCTION

1.1 Rationale for study

The Cape Floristic Region (*sensu* Goldblatt & Manning 2000; hereafter referred to as the CFR; Figure 1.1), an area of about 90,000 km^2 situated at the south-western tip of Africa, is renowned for its remarkable floristic richness and narrow-range endemism. Encompassing approximately 9,000 species, the region's floristic diversity is comparable with that of Neotropical rainforests (Goldblatt & Manning 2002; Linder 2003), and distinguishes the CFR as one of the world's biodiversity hotspots (Myers *et al.* 2000). Being bordered by deserts (Nama- and Succulent Karoo) and oceans (Indian and Atlantic Ocean), the region is biogeographically and physiographically isolated. As a consequence, the CFR shows strong floristic endemism, especially at the species and genus levels (69% and 17%, respectively), with much of the floristic diversity having arisen *in situ* (Goldblatt 1978; Cowling *et al.* 1996; Goldblatt & Manning 2002; Linder 2003, 2005). The vegetation is dominated by fynbos, renosterveld and strandveld (Fynbos biome; *sensu* Mucina & Rutherford 2006), the distributions of which are closely associated with variation in soil type and rainfall regime (Boucher & Moll 1981; Thwaites & Cowling 1988; Cowling & Holmes 1992; Goldblatt & Manning 2002). Despite extensive research on various aspects of the Cape flora and its environment, the origins of the flora and its diversity remain poorly understood, not least because the region lacks a detailed reconstruction of its palaeoenvironments. Attempts at resolving the finer details of this have been undermined by a scarce fossil record (Scott 1982; Coetzee 1983) and by the poor resolution for the region's geomorphological evolution. Furthermore, the heterogeneity and scale-dependent structuring of the region's habitat mosaic, extending from local to regional scale, introduce additional challenges to reconstructing the region's palaeoenvironmental and floristic evolutionary history.

The physical complexity of the CFR is determined by its high landform diversity, with steep mountains, valleys and coastal plains capped by a mosaic of soil types (Lambrechts 1979; Deacon *et al.* 1992). Moreover, distinct climatic gradients characterize the CFR. Regionally, there is an East-West gradient in the intensity of summer-aridity as part of the winter-rainfall climatic regime (Tyson 1987), while on a more local scale, differences in moisture availability occur along altitudinal gradients (Marloth 1904; Fuggle & Ashton 1979).

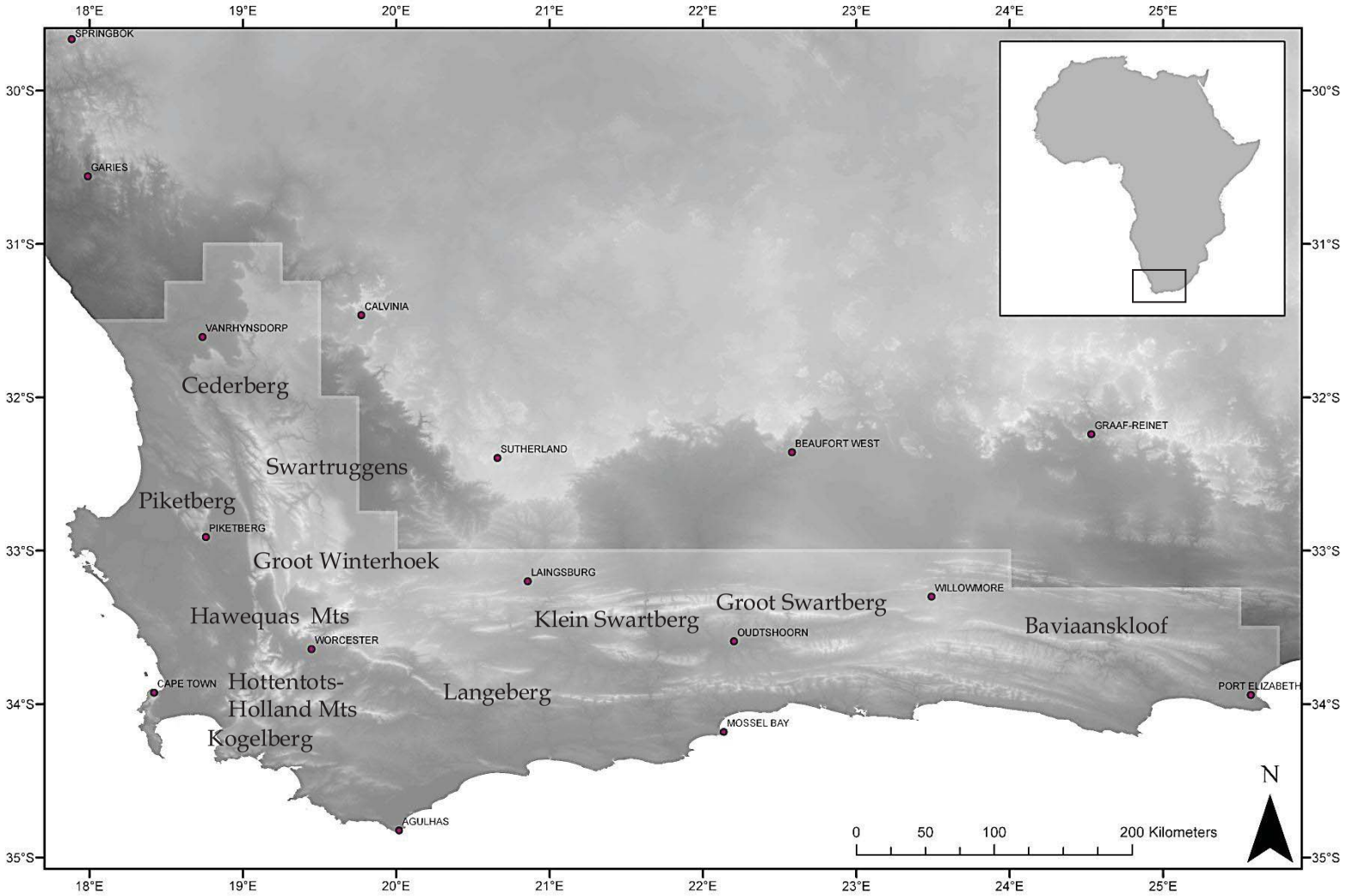

Figure 1.1 Outline of the Cape Floristic Region (CFR, *sensu* Goldblatt & Manning 2000), and the major mountain ranges of the Cape Fold Belt (CFB).

This physical heterogeneity is commonly invoked as an explanation of the region's floristic diversity, on account of its potential role as a driver of ecological divergence and, ultimately, speciation (Verboom *et al.* 2003, 2004; Hardy & Linder 2005, 2007; Latimer *et al.* 2009; van der Niet & Linder 2009; Carlson *et al.* 2011). Furthermore, the nature and diversity of the flora are believed to have been strongly influenced by historical changes in the physical environment. On the one hand, a relatively stable climate (Jansson & Dynesius 2002) and mild tectonic perturbation (Hendey 1983) of the region are thought to have allowed for the gradual accumulation of diversity over time (Linder 2003). On the other hand, increasing aridity and the establishment of a seasonally-arid climate regime (Levyns 1964; Goldblatt 1978; Linder 2003) and, perhaps, neotectonic uplift since the mid-Miocene (Cowling *et al.* 2009) are suggested to have stimulated recent radiation of the flora. The quest to understand the origins of this flora and to tease apart the relative roles of long-term stability and recent environmental modifications of the landscape on its evolutionary history, however, requires the foundation of reliable knowledge of the region's palaeoenvironment.

A novel approach to refining details of palaeoenvironmental evolution in the CFR is to employ geobiological evidence, which resides in the genomic record of selected biotic indicators (Cotterill & de Wit 2011). The genomic record preserves evidence for the origin and evolution of indicator species as these track their preferred habitat or niche-space across time and space. Ecological specialists, confined to narrow niches, are particularly informative. This asymmetrical role of stenotopes invokes the 'effect hypothesis' of macroevolutionary dynamics, which argues that ecological specialists exhibit higher sensitivity to palaeoenvironmental impacts (Vrba 1980). Such geobiological evidence provides us with a proxy to decipher the co-evolutionary trajectories of biota and landform. These historical signatures can provide a novel insight into macroevolutionary trends and palaeoenvironmental evolution, especially in landscapes whose environmental history is poorly understood, and has proved intractable to conventional methods in geology and palaeoclimatology. The way in which such phylogenetic information has provided insights into biome evolution (Pennington *et al.* 2004; Verboom *et al.* 2009), drainage evolution (Goodier *et al.* 2011) or into historical aspects of ecosystems such as fire (Bytebier *et al.* 2011; Simon *et al.* 2009) introduces the possibility of refining the spatio-temporal resolution of models of palaeoenvironmental evolution for the CFR. The 'geoecodynamic' approach, which employs the genomic record to pinpoint the divergence of populations or lineages in

time and space (Cotterill & de Wit 2011), provides a means of refining the spatio-temporal resolution. The feasibility of using this geoecodynamic approach rests on the assumption that many taxa of Cape plants are tightly constrained to particular habitats in the landscape mosaic, i.e. there is a high incidence of stenotopy. Since many lineages of Cape plants occupy a diversity of habitats, including those habitats that resemble more closely presumed early-Miocene conditions, there exists an opportunity to use these lineages to evaluate which aspects of the region's physical mosaic reflect conditions in deeper time (> 10 Ma) and to track changes that have taken place subsequently. This thesis attempts to do this, using phylogenetic and geospatial data for about 450 plant species (~5% of the Cape flora) belonging to 11 distinct lineages. Before articulating the key hypotheses of this thesis, I briefly outline the climatic and geomorphic history of the region, as well as current theories pertaining to the origin of the region's floristic diversity.

1.2 Cape climates

Typically described as 'Mediterranean', the climate of the CFR contrasts sharply with the summer rainfall climate that prevails over the rest of southern Africa (Coetzee 1978a; Tyson 1987). There is, however, a distinct longitudinal trend in rainfall seasonality within the CFR: while the western half of the region endures a strongly seasonal rainfall regime in which most rainfall occurs during the winter months, the eastern half of the CFR experiences a more aseasonal (or bimodal) rainfall regime (Tyson 1987). Most rainfall in the extreme south-west and west is associated with the north-westerly cold front events that reach the region in winter (June –September) under the influence of the South Atlantic anticyclone system (Tyson 1987). Summers here are characterized by long dry spells, attributable to the drying effect of the Benguela Upwelling System (BUS) and anticyclonic conditions (Coetzee 1978a; Tyson & Partridge 2000). In contrast, the eastern part of the Cape region (from Cape Agulhas to Port Elizabeth) receives rainfall during spring and autumn associated with moist air from the warm Indian Ocean that is regularly advected towards the eastern CFR by high-pressure cells (Coetzee 1978a; Cowling & Holmes 1992). Mean annual rainfall is highest in the region of the syntaxis (or south-western mountains, Goldblatt 1978) and around Knysna in the East, and is lowest in the west and further inland (see also Figure 2.2).

The topography of the landscape also influences localized climatic conditions, such that these deviate from the regional-scale patterns described above in two main ways. As a consequence of the distinct east-west trend of the CFB parallel to the coastline, a rain shadow is created along the northern, interior mountain slopes, resulting in north-facing, slopes being drier than the south-facing slopes (Goldblatt & Manning 2002). Arguably more important, however, is the impact of orographic precipitation in the montane environments by means of wet stratus clouds that frequently cover mountain peaks (Marloth 1904; Nagel 1962, 1965; Fuggle & Ashton 1979; Goldblatt & Manning 2002). Their formation is driven by the region's characteristic south-easterly trade winds which are strongest in summer when the aridity is most severe. While these clouds may not necessarily precipitate rain, their importance as a source of moisture for the Cape montane biota should not be underestimated, potentially matching the importance of fog as a source of moisture in deserts (Namaqualand, Vogel *et al.* 2011) and other ecosystems (California redwood forest, Dawson 1998). Hence, summer aridity at higher altitudes is probably less acute than at lower altitudes, especially in the more seasonally-arid western part of the CFR.

Climatic reconstructions based on both fossil and global oxygen isotope records suggest that the climate of the region was historically more sub-tropical than it is today (Coetzee 1978a, b; Tyson 1987; Partridge 1993; Zachos *et al.* 2001; Dupont *et al.* 2011). On a global scale, the succession from a tropical to a more temperate climate in the CFR reflects a global cooling trend since the Eocene that bears witness to world-wide impacts on the structure and composition of biotic communities (Prothero 1994). From a more regional perspective, however, climatic perturbations driven by a combination of anti-cyclonic high-pressure cells, coastal upwelling and possibly tectonic uplift are likely to have stimulated local vegetation turnover and impacted the evolution of the modern Cape flora.

Changes in the geometry and inter-connectivity of ocean basins that accompanied the break-up of Gondwanaland since the Cretaceous are likely to have played a key role in the global cooling trend evident since the Early Eocene Climatic Optimum (Lagabrielle *et al.* 2009). The opening of the Drake Passage as well as the Tasman Gateway during the Eocene led to the development of a deep ocean circulation system, the Antarctic Circumpolar Current (ACC), around a recently isolated Antarctic continent that had been in a polar position since the Late Cretaceous (Kennett 1977; Tyson 1987; Scher *et al.* 2006; Livermore *et al.* 2007). In response to this, the Late Eocene formation of ephemeral ice sheets and the

permanent glaciation of the newly isolated Antarctic continent at the start of the Early Oligocene (Kennett 1977; Zachos *et al.* 2001) would have enhanced global cooling significantly (Scher *et al.* 2006; Livermore *et al.* 2007). The oceanic circulation system that was established at this time is likely to have been a pre-cursor to today's thermo-haline circulation system that re-distributes heat across the globe and drives the world's modern climate systems**.** The establishment of the ACC arguably had magnified consequences for the Cape region in that it may have led to the formation of an early, 'proto'-Benguela current, which, in turn could have invoked localized drying effects on land (Lagabrielle *et al.* 2009). The degree to which this would have influenced vegetation patterns at that time, however, is largely unknown, not least because most of the fossil pollen assemblages from the West coast are restricted to the Late Cenozoic era (Coetzee 1978a, b; Dupont *et al.* 2011).

In southern Africa, and more specifically in the CFR, three major events since the Early Miocene are considered to have played an important role in the evolution of the modern flora; (i) the establishment of the BUS during the Mid-Miocene and associated aridification as part of the global cooling trend (~10-14 Ma; Siesser 1980; Zachos *et al.* 2001; Diester-Haass *et al.* 2002; Dupont *et al.* 2011), (ii) uplift-induced aridification at the Miocene/Pliocene boundary (~ 5 Ma; Tyson & Partridge 2000; Linder 2003), and, most recently, (iii) the onset of the Plio-Pleistocene glacial period (~ 3 Ma; Coetzee 1978a, b; van Zinderen-Bakker & Mercer 1986).

Despite the switch from a global 'greenhouse' to a global 'ice house' at the Eocene/Oligocene boundary (see Prothero 1994), it is not until the Mid-Miocene that large-scale aridification of southern Africa is likely to have taken place (Coetzee 1978a, b; Siesser 1980; van Zinderen-Bakker & Mercer 1986; Partridge 1993; Dupont *et al.* 2011). This trend is also evident from the global climate record (Zachos *et al.* 2001). Several lines of evidence suggest that the onset of upwelling (or strengthening of south-easterly winds) dates back to this time (Siesser 1980; Diekmann *et al.* 2003; Krammer *et al.* 2006; Dupont *et al.* 2011). This upwelling system of the cold, north-flowing Benguela current is invoked as the determinant of the arid and hyper-arid climates of south-western Africa (Tyson 1987; Partridge 1998). This, in turn, is driven by south-easterly winds activated by the South Atlantic anticyclone high-pressure system which has a far-reaching drying effect by inhibiting the westward flow of moist tropical air (Goldblatt 1978; Tyson 1987), as seen along the West coast and most prominently in the Namib Desert (Coetzee 1978a, b; Siesser 1980). In addition, it has been

argued that aridification, especially of the western CFR, could also reflect neotectonic uplift in the late Neogene (Tyson & Partridge 2000; Linder 2003). Uplift of the escarpment edge along the eastern part of southern Africa is argued to have produced a 'rain shadow', whereby the higher mountains in the East effectively intercept tropical moisture, intensifying summer-aridity in the West. Hence, while the establishment of the BUS during the Mid-Miocene is likely to have been instrumental in the aridification of the CFR, subsequent tectonic uplift would have intensified arid conditions (Tyson & Partridge 2000; Linder 2003; Linder *et al.* 2006). Evidence for uplift-induced aridification is, however, conspicuously lacking. Nevertheless, invoking evidence from South America, Cracraft (1985, 1992) argued that tectonics is the ultimate determinant; by generating landform heterogeneity, tectonics has driven allopatric speciation and extinction. In this context, Partridge *et al.* (1995) suggested that the significant epeirogenic uplift of southern and east Africa was the ultimate cause of biotic diversification through the late Cenozoic.

Most recently, southern African climates are thought to have been influenced by pronounced fluctuations between glacial and interglacial periods through the Plio-Pleistocene (Tyson 1987; Partridge 1997; Lisiecki & Raymo 2005, 2007; Chase & Meadows 2007). These episodes were characterized by the growth and retreat of the polar ice-sheets, particularly in the Northern Hemisphere, and are driven by orbital forcing (Hays *et al.* 1976; Deacon *et al.* 1992; deMenocal 2004; Lisiecki & Raymo 2005). Their direct impact on biotic communities and assemblages is generally believed to have differed across arctic, temperate and tropical latitudes (Hewitt 2000, 2004). While Brain (1982, 1985) and Vrba (1985, 1999) invoked Plio-Pleistocene climate changes as the primary cause of mammalian evolution across Africa, impacts of these switches between ice sheet expansions and contractions on southern African biodiversity are not well understood , especially in the Cape. Even during the coldest periods of the Pleistocene the highest peaks in the Cape Mountains are likely to have been below the permanent snowline (Deacon *et al.* 1992). The fauna and flora here would thus have been impacted by increased aridification and shifts in rainfall regimes (Chase & Meadows 2007) associated with ice sheet expansion rather than by large-scale lineage extinction as observed in the high latitudes of the northern hemisphere. Furthermore, mountainous regions such as the CFR are likely to have played an important role as climatic refugia for species, allowing lineages to 'track' their climatic niche along an altitudinal gradient as climates fluctuated (Hewitt 2000, 2004). Hence, the steep, yet

moderately high mountains of the Cape have probably acted rather as refugial, climatically-stable environments that allowed plants to persist throughout the climatic fluctuations (Jansson & Dynesius 2002).

A detailed reconstruction of the Cape palaeoenvironment since the Oligocene/Miocene has been largely confounded by the scarcity of plant fossil sites and by the lack of fine-scale palaeoclimatic data (Coetzee 1983; Scott 1995; Linder 2003; Cowling & Proches 2005; Chase & Meadows 2007). Nevertheless, the pollen fossil record has served as an invaluable source of data for reconstructing the palaeofloristic turn-over at the regional scale, serving as a platform for palaeoclimatic inference (Coetzee 1978a, b, 1983; Scott 1982; Dupont *et al.* 2011). For example, the fossil record suggests that the predominance of the modern flora, dated to the Pliocene (Coetzee 1978b), is decoupled from the origins of many of its characteristic and dominant groups (Restionaceae, Proteaceae, Ericaceae, Bruniaceae), which date back to Cretaceous times (Linder 2003; Sauquet et al 2009; Edwards & Hawkins 2007; Quint & Classen-Bockhoff 2008). Yet, it has failed to depict in more detail the intricacies of palaeoenvironmental evolution in a complex and floristically diverse landscape. This is partly because fossil pollen data for the CFR are scarce, provide information only about the presence and relative abundance at the family- and genus level, and, most importantly, because their low spatial fidelity provides limited resolution on past distributions of lineages (Scott 1982; Coetzee 1983). Moreover, Linder (2003) questioned the validity of extrapolating inferences applied from fossil pollen records obtained from offshore or coastal sediment cores to both montane and lowland regions, which might be particularly problematic in a topographically-complex environment such as the CFR.

1.3 Cape topography and landscapes

The Cape Fold Belt (hereafter referred to as the CFB) is a thrust-and-fold mountain belt located below the Great Escarpment along southern Africa's south-western margin (Dingle *et al.* 1983; Newton *et al.* 2006). This mountain belt forms a distinct series of prominent ridges that run parallel to the southern and western coastlines. Their persistence is attributed to their highly erosion-resistant quartzitic sandstone lithology (Cape Supergroup: Table Mountain, Bokkeveld and Witteberg Groups). These ridges are interspersed by intermontane valleys and gently undulating coastal plains that are characterised by

moderately fertile soils derived from the more fine-grained rocks of Pre-Cambrian origin (i.e. mostly Malmesbury Group; Cowling *et al.* 2009).

The Cape Supergroup sediments were deposited in a passive margin setting (the Cape Basin) across an ancient erosion surface from the Early Ordovician to the Early Carboniferous (Thamm & Johnson 2006), ceasing at the end of the Permian with the onset of the Cape Orogeny (McCarthy & Rubidge 2005; Johnston 2000). Thereafter, over a period of approximately 65 million years, the sedimentary rocks of the Cape Supergroup were episodically folded and faulted by compressional forces as a subduction zone developed along the southern margin of Gondwana (280 to 215 Ma), giving rise to the modern CFB.

Structurally, the CFB consists of two main zones of folding that converge around the Ceres-Worcester-Hermanus region to form the Cape Syntaxis (Dingle *et al.* 1983; Newton *et al.* 2006). North of the syntaxis, the western arm of the CFB (Table Mountain Group, TMG) stretches along a north-south axis with folds curving concave to the west (Olifants River, Cold Bokkeveld, Cederberg; Lambrechts 1979). To the East, the TMG is replaced by the Witteberg quartzites of the Swartruggens range, and by the east-west trending coastal and inland ridges of the Langeberg, Swartberg and Baviaanskloof mountain ranges (Fig 1.1). Of steep relief but moderate altitude, these ancient mountains attain a maximum elevation of 2325 m in the East (Seweweekspoort) and 1995 m in the West (Du Toit's Peak). The Cape Syntaxis is a north-east trending structural domain where folding follows various directions (de Beer 1995; Johnston 2000; Newton *et al.* 2006), and extends south-west across the Hex River, Du Toit's, Hottentots Holland and Kogelberg mountain ranges to the coast (Lambrechts 1979).

The CFB facies are largely quartzitic and produce soils that are sandy, base-poor (acidic) and therefore relatively infertile (Kruger 1979; Witkowski & Mitchell 1987). The fine-grained sandstone units of the Bokkeveld Group differ in being interspersed with mudrock units, while the Cederberg and Pakhuis Formations consist largely of shales, diamictites and mudstones (Thamm & Johnson 2006; Cowling & Holmes 1992). These shale-strata of the Cape Supergroup weather into clay-rich soils that form comparatively fertile, loamy patches set in amongst the typically nutrient-impoverished, sandy soils of the CFB.

The 'Coastal Foreland' extends from the lower seaward slopes (< 300m) of the CFB to the coastline (as described by Wellington 1955). These smoothly undulating plains are underlain

primarily by shales and phyllites of the basement inliers of the CFB stratigraphy, and reach a maximum elevation of approximately 200-300 m (Lambrechts 1979). These inliers consist of a suite of fine-grained Precambrian sediments (Malmesbury, Kaaimans, Gamtoos and Kango Groups) which were intruded by granites (Cape Granite Suite) towards the end of the Precambrian (Scheepers & Armstrong 2002; McCarthy & Rubidge 2006). Exposure of these sediments is manifest in valleys and coastal plains, and gives rise to fine-grained soils which contrast with the relatively infertile sandstone-derived soils of the mountains.

Directly along the coast, aeolian and marine deposits dating from the Neogene dominate the landscape, and in some parts, form extensive dune fields or raised calcareous platforms (see Roberts 2009). On the one hand, the extent and exposure of these calcareous deposits would have been influenced directly by sealevel fluctuations throughout the history of the region, at times exposing (and inundating) large expanses of coastal shelf. This was most extensive along the Agulhas Plain (Siesser & Dingle 1981; Compton 2011). Conversely, neotectonic uplift of the southern African continent has arguably played an important role in the formation of calcareous deposits by raising coastal platforms, especially along the south coast (McMillan 1990; Roberts & Brink 2002). So it appears that the formation and emergence of calcretes (often incorrectly classified as limestones in the botanical literature) along the South Coast, reflects impacts of both marine regressions/transgressions and tectonic uplift.

The history of the CFR landscape has evidently been complex, as reflected by the diversity of coastal and terrestrial landforms, differing in age, aspect and lithology (Wellington 1955; de Wit 2007; Partridge *et al.* 2010), and whose evolution has been influenced by events on both a local and a regional scale (subcontinental). In tandem with climatic changes during the Cretaceous, the break-up of Gondwana forged the southern margin of Africa along which the erosion-resistant sandstone ridges of the CFB have since undergone erosion (and exhumation) to form the modern Cape landscape (Lambrechts 1983; Tinker *et al.* 2008a, b). Thereafter, the region is thought to have been relatively stable throughout the Cenozoic (Hendey 1983; Tinker *et al.* 2008a, b). Yet the CFB and adjoining coastal plain display two striking landscape features, whose anomalous elevation testifies to at least one episode of relatively recent vertical uplift that affected the entire southern margin of Africa (Haughton 1969; Truswell 1977). Firstly, the disproportionately deeply-incised river gorges (Storms River, Great Fish, Kei, Sundays River) across the south-eastern mountain ridges cannot be

attributed solely to the action of fluvial erosion (Haughton 1969). Secondly, wave-cut terraces are preserved at relatively high altitudes along the western and southern expanses of the coastal plain, and are unlikely to be the sole result of marine transgressions (Haughton 1969; McMillan 1990).

In their seminal paper on the evolution of southern African landscapes, Partridge and Maud (1987) argue that such signatures of uplift in the CFB have been the result of punctuated tectonic uplift events that centred along the Ciskei-Transkei flexure axis during the Miocene with far-reaching effects on most of southern Africa (see Figure 1.2; axes according to Partridge & Maud 1987; Moore 1999). While initial uplift at the beginning of the Miocene was negligible (~200 m), subsequent uplift at the Miocene/Pliocene boundary was arguably more pronounced (~900 m; Partridge & Maud 1987, 2000; Partridge 1997; Partridge *et al.* 2010). This regional epeirogenic event has been invoked as a possible trigger for the diversification of the Cape flora (Cowling *et al.* 2008), especially where it caused increased erosion and exhumation of underlying bedrock.

This model of neotectonic uplift has, however, been criticized on a number of grounds. These primarily relate to the model having been inferred largely from anecdotal evidence using traditional qualitative geomorphological methods (i.e. pediplanation, correlating erosion surfaces), whose utilization is questionable and requires critical assessment (Truswell 1977; see also van Wateren & Dunai 2001). Attempts at quantitatively classifying land surfaces of the CFB using fine-scale Shuttle Radar Topography Mission (SRTM)-derived data in order to recover empirical evidence for Partridge and Maud's erosion surfaces (1987) have been unsuccessful. Hence empirical evidence in support of the model is lacking, at least at a regional scale (Mielke 2008). Hence, the extent and timing of neotectonic uplift of southern Africa remains to be rigorously evaluated.

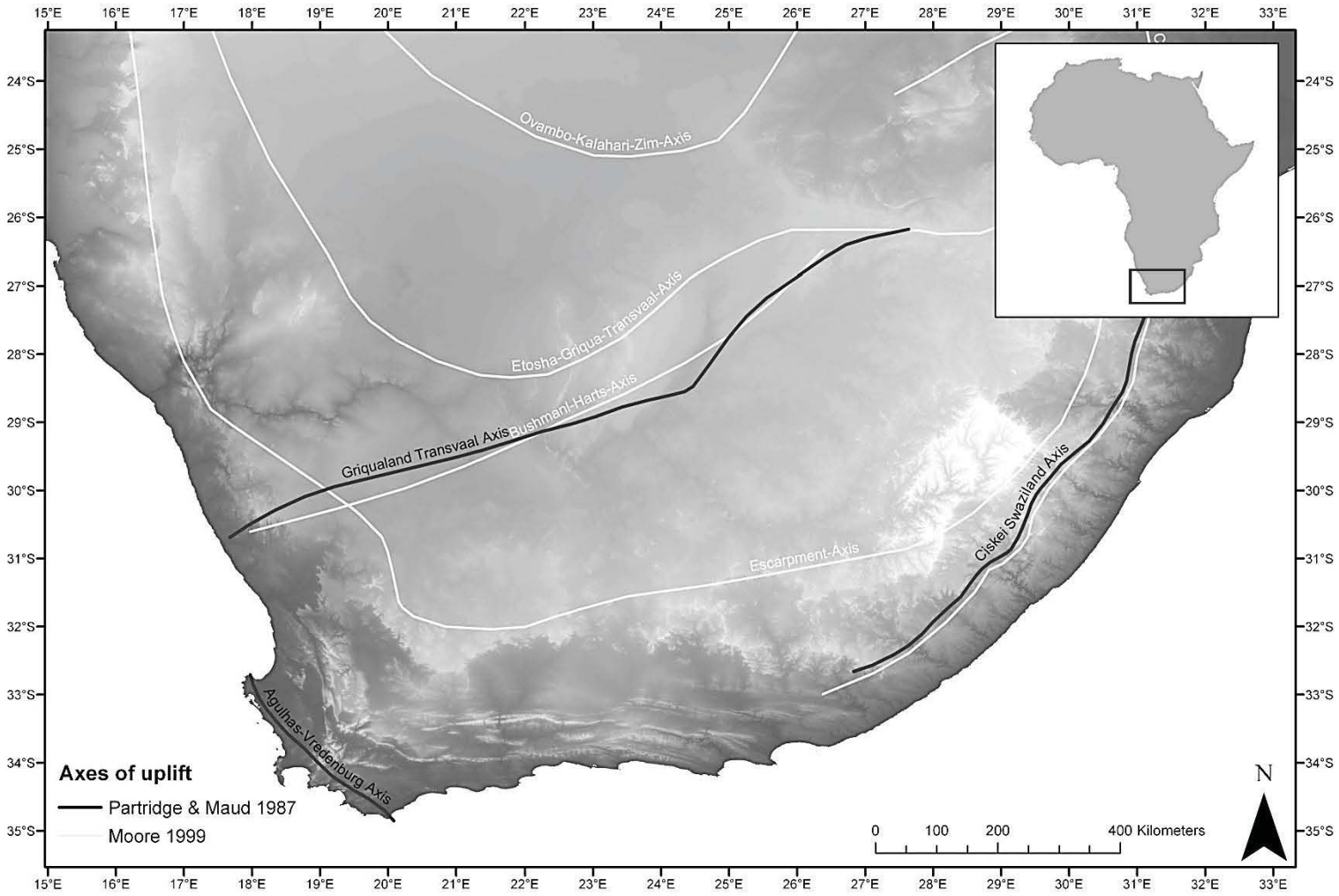

Figure 1.2 Digital Elevation Model (DEM) of southern Africa with the different flexure axes along which tectonic uplift has been proposed to have occurred during the Miocene. In the context of landscape evolution in the Cape Floristic region (CFR), the Ciskei-Swaziland Axis (Partridge & Maud 1987; Moore 1999)has been of most relevance to neotectonic uplift of the region.

While there is ongoing progress to address these shortcomings, the current state of geomorphological knowledge fails to provide a coherent account of the ages and episodes of landform evolution in the Cape. This deficiency contrasts against the regional understanding refined for southern Africa over the past decade (Moore 1999; Moore *et al.* 2009; de Wit 2007; Tinker *et al.* 2008a, b; Decker 2010; Decker *et al.* 2011). The lack of quantitative estimates of denudation rates, as provided, for example, by studies of low temperature thermochronology (UTh/He; Lisker *et al.* 2009), has hindered the development of a reliable reconstruction of the geomorphic history of the Cape region. Furthermore, due to the absence of volcanic activity in southern Africa since the Eocene at least, the region lacks rocks which are amenable to radiometric dating which might serve to constrain exposures and/or emergences of specific land surfaces (Partridge 1998). Such quantitative estimates are a prerequisite for robust reconstructions of the tempo and mode of landscape evolution over time scales of $10^6 – 10^8$ years and at spatial scales of $10^2 – 10^3$ m (mesoscale). However, the quest for such estimates lies beyond the practicable scope of current geomorphological methods. Thus, low-temperature thermochronology, and even cosmogenic measurements can only quantify average rates of denudation within a river valley or across an eroding plateau at the meso- and macroscale. Low spatial fidelity inherent to these methods is especially restrictive, where it is rare to be able to place precise dates on individual landforms (Gregory 2010; Cotterrill & de Wit 2011). A way forward to unravelling local-scale geomorphology is to adopt the geoecodynamic approach (Cotterill & de Wit 2011). Central to this approach is the concept of stenotopy as well as the 'molecular clock' which define spatial boundaries and temporal events in the evolution of indicator species, respectively. By being intimately coupled to climatic and edaphic niches, stenotopes hold valuable information about the emergence and spatial shifting of these niches as inscribed in their genomic record over time. Here, such an approach may aid in unravelling the geomorphological evolution of the CFR by tracking and reconstructing the tempo and mode of the evolution of substrate-endemic taxa across a number of representative plant clades.

1.4 The radiation of the Cape flora

The climatic and geomorphic changes described above are likely to have stimulated the evolutionary radiation of the flora both by stimulating adaptive divergence in the seasonally-

arid lowland plains (Levyns 1964; Linder *et al.* 1992) and by fragmenting the ranges of formerly more continuously-distributed species across the relatively mesic mountains (Adamson 1958; see also Smith *et al.* 2001).

Under an adaptive radiation model, rapid lineage diversification is accompanied by phenotypic and/or ecological divergence facilitated by the 'opening up' of a novel adaptive zone, which, in turn, is driven either by abiotic perturbations or by key innovations (Simpson 1944; Schluter 2000). In the CFR, drastic climatic deterioration during the Miocene and Pliocene is proposed to have precipitated widespread extinction of the pre-existing flora, probably a palm-dominated subtropical-tropical vegetation (Coetzee 1978a, b; van Zinderen-Bakker & Mercer 1986), which was arguably poorly adapted to cope with drought. This generated a vacant adaptive zone (sensu Simpson 1944), especially in the lowlands, which became available for colonization by suitably pre-adapted lineages (Levyns 1964; Linder *et al.* 1992), such as those comprising the Cape flora. According to Levyns, elements of this flora had the necessary pre-adaptations needed to enable it to survive protracted seasonal aridity (*Phylica*, Richardson *et al.* 2001; *Ehrharta*, Verboom *et al.* 2004). Furthermore, geomorphological rejuvenation of the Cape region, beginning in the Early Miocene has been invoked as a trigger for floristic diversification by means of adaptive radiation (Cowling *et al.* 2009). Driven by episodes of Neogene tectonic uplift and marine transgressions, the exposure of shale (and to a lesser degree granite) strata and coastal deposits, respectively, is suggested to have opened up a novel adaptive zone within a relatively homogenous infertile quartzite-derived soil and silcrete-capped landscape, especially in the lowlands (Partridge & Maud 1987; Cowling *et al.* 2009). Models of uplift-induced floristic radiation have been suggested in other parts of the world (Quinghai-Tibet, Wang *et al.* 2009), but whether or not this re-sculpturing of the Cape landscapes simply provided the landscape heterogeneity required to promote the climate-driven radiation within a newly formed, seasonally-arid niche in the lowland plains remains to be explored.

Lineage divergence driven by non-adaptive processes is also likely to have played an important role in the radiation of the Cape flora, in particular across the CFB mountains. In contrast to adaptive radiation, non-adaptive radiation is typically associated with negligible phenotypic and/or ecological differentiation between diverging lineages (Kozak *et al.* 2006; Rundell & Price 2009). In the CFR, climatic deterioration since the Mid-Miocene may have promoted the radiation of lineages by means of range fragmentation and allopatric

speciation of a once-continuous Cape flora (Adamson 1958; see also 'sky-island' effect, Roy *et al.* 1997; Knowles 2001). The combined influences of complex topography and climatic fluctuations could have created and maintained islands of mesic, sandstone habitats across the Cape landscape that were environmentally similar but geographically isolated (i.e. mountain peaks). This archipelago would have provided spatially fragmented refugia where lineages were able to persist despite climatic fluctuations, in a similar manner to the 'refuge' model of speciation (Kozak & Wiens 2007). Here, relative climatic stability would have allowed the persistence and accumulation of species over time (*sensu* Jansson & Dynessius 2002). In the case of this fragmentation model, fire may also have played a role as a fragmenting agent. Fire is recognized as a dominant control over the composition of the Cape vegetation across contemporary timescales (Cowling 1987; Bond & van Wilgen 1996; Ojeda et al. 2005) and its evolutionary importance in lineage divergence have been recognized (Segarra-Moragues & Ojeda 2010; Bytebier *et al.* 2011). Whether or not the fragmentation of communities and populations in response to fire is, on a macro-evolutionary scale, sufficiently influential to inhibit gene flow effectively over several generations and thereby promote cladogenesis remains debatable (Barraclough 2006). In CFR, the patchy incidence of fire may simply have exacerbated the ultimate impact of climatically- and topographically-driven fragmentation.

The evolution of the CFR's floristic diversity has evidently been complex, likely having been driven both by means of adaptive and non-adaptive processes (Verboom *et al.* 2009), each of these mechanisms having operated in different parts of the region. Lowland environments, especially in the West, most strongly reflect the novel arid-adaptive zone suggested to have prompted adaptive radiation since it is here that seasonal aridity is most pronounced. By contrast, seasonal aridity in the montane, topographically complex environments is probably less severe due to moisture input by the southeaster cloud belt. Hence, while the mountains may have acted as climatic refugia especially during the Plio-Pleistocene glacials (*sensu* Jansson & Dynessius 2002), climatic conditions in the lowland plains were probably much harsher and unstable. Based on this, it may be predicted that diversification of the upland/refugial aseasonal flora followed a non-adaptive 'fragmentation' model (Adamson 1958), while diversification in the edaphically-heterogeneous and seasonally-arid lowland plains may have conformed more closely to a Simpsonian adaptive radiation model, as envisaged by Levyns (1964).

One of the key features of adaptive radiations is significant phenotypic differentiation between diverging lineages as these adapt across an array of different environments, i.e. within a novel 'adaptive zone' (Schluter 2000). In contrast, phenotypic and ecological differentiation across diverging lineages is generally negligible in non-adaptive radiations (Kozak *et al.* 2006; Rundell & Price 2009). This is because under the latter model, speciation is typically driven by the inability of lineages to adapt to novel environments ('phylogenetic niche conservatism', Wiens & Graham 2005; Wiens *et al.* 2010), leading to range fragmentation, isolation and, ultimately, allopatric speciation as climatic changes lead to spatial shifts of the preferred niche-space. Hence, the degree of phenotypic differentiation is generally predicted to be higher in lineages diverging within a novel adaptive zone (depicted as the straight line in Figure 1.3). In the context of the Cape environment, then, in which the onset of seasonal aridity is thought to have triggered the radiation of the Cape flora, we would expect to find comparatively high levels of phenotypic differentiation between sister lineages that diverged in the strongly seasonal, edaphically-heterogeneous lowlands. In contrast, relatively low levels of phenotypic divergence are expected amongst lineages that radiated non-adaptively in the aseasonal, montane habitats. This relationship is, however, better evaluated by incorporating overdispersion since not all factors responsible for a particular pattern are incorporated as predictor variables in ecological/evolutionary models (depicted as the grey-shaded triangle in Figure 1.3; Cade *et al.* 1999; Cade & Noon 2003). Hence, the adaptive and non-adaptive processes that generated species complexes will be mirrored in their degree of phenotypic and ecological divergence (Schluter 2000; Rundell & Price 2009). Quantitative explorations of the degree of morphological divergence in a set of vegetative traits across the seasonality gradient, representing the full adaptive/non-adaptive zone could then reveal the relative roles of these respective processes in the Cape radiation. In conjunction with diversification rate analyses, which are used to identify clades that have experienced rapid speciation rates, this is a potentially powerful approach to understand the roles of these processes at a biome scale.

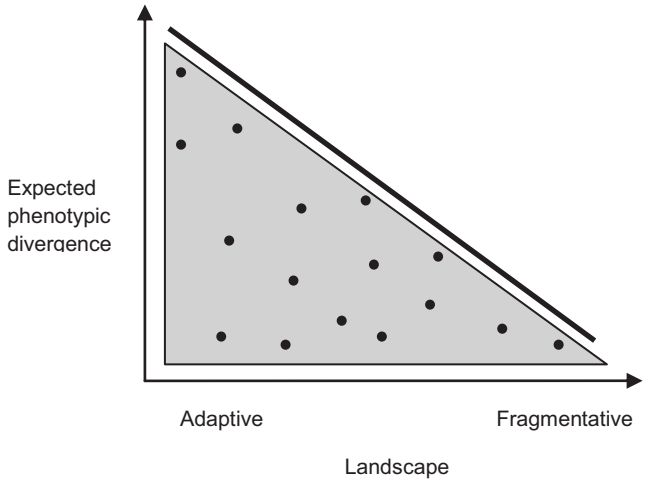

Figure 1.3 Illustrative graph of the expected 'triangular' relationship in the degree of phenotypic divergence across two contrasting selective landscapes. In the case of the CFR, the adaptive and fragmentative landscapes are represented by seasonal and aseasonal habitats, respectively. Adaptive radiations (by means of ecological speciation) are accompanied by high phenotypic differentiation (Schluter 2000). Non-adaptive radiations (by means of allopatric speciation), on the other hand, create species that are ecologically and phenotypically similar as lineages diverge in geographic isolation (Rundell & Price 2009).

1.5 Aims and hypothesis

The central aims of this thesis are (i) to test the feasibility of using elements of the flora as 'bioindicators' to track the environmental history of the Cape, both geomorphological and climatic, and (ii) to obtain an improved understanding of the environmental conditions (ancestral versus novel) or habitats that supported different adaptive versus non-adaptive processes in the radiation of the Cape flora. The accumulation of relatively densely-sampled phylogenies of Cape clades over the past few decades positions us to test the geoecodynamics research strategy in the novel context of the Cape region using multiple phylogenetic data sets. By reconstructing trends in the evolution of habitat specialization (seasonality, rainfall, and substrate) across several molecularly dated phylogenies, it becomes possible to draw general inferences about the evolution of the Cape environment, and better understand how this has shaped the modern flora.

This ‘multi-lineage’ approach was applied here to test the following hypotheses:

(1) High-altitude, aseasonal habitats on sandstone-derived soils represent the ancestral habitats, while the occupation of low-altitude, seasonally-arid habitats on shale and/or calcareous substrates is a derived feature in Cape lineages.

(2) The transition towards seasonal-, arid-endemism coincides with the onset of a seasonally-arid conditions 10 - 14 Ma in response to the establishment of the Benguela upwelling system (Siesser 1980; Levyns 1964). Alternatively, such transitions coincide with uplift-induced intensification of seasonal aridity about 5 Ma (Linder 2003).

(3) The transition to more fertile shale-derived soils coincides with Pliocene tectonic uplift, associated increased erosion and subsequent exposure of the underlying shale strata around 3 - 5 Ma ago (Cowling *et al.* 2009). Similarly, the occupation of calcareous coastal substrates coincides with this Pliocene uplift.

(4) Climatic changes precipitated increased floristic diversification, with diversification in aseasonal, climatically stable (montane) and seasonal, edaphically-heterogeneous (lowland) environments proceeding via non-adaptive and adaptive mechanisms, respectively, resulting in differential morphological divergence between lineages (Figure 1.3).

CHAPTER 2: MATERIALS AND METHODS

2.1 Taxon sampling

The following criteria were employed for the selection of plant clades (see Table 2.1 for a list of selected clades):

(i) The availability of a published phylogeny and an associated DNA sequence matrix. In cases where DNA matrices were not available on TreeBASE, they were obtained directly from the respective authors.

(ii) Adequate species-level sampling of the given clade (at least >70%). The effect of missing taxa especially on the precision and accuracy of ancestral state reconstruction analyses (Schultz & Churchill 1999; Salisbury & Kim 2001), as well as phylogenetic and molecular dating analyses, is well documented (Sanderson & Doyle 2001; Linder *et al.* 2005; Pirie *et al.* 2005; Heath *et al.* 2008).

(iii) Clades should be composed largely of taxa that are geographically restricted to the CFR.

(iv) Clades with high environmental niche diversity are most likely to provide insight into the palaeoenvironmental and floristic evolution of the edaphically and climatically heterogeneous CFR. Hence, selected clades should comprise taxa that occupy diverse environments.

Finally, the aim was to include clades representing a phylogenetically diverse sample of the dominant families of the Cape flora, i.e. Asteraceae, Orchidaceae, Restionaceae, Proteaceae, Poaceae and Cyperaceae. A brief description of the current taxonomic status and ecologies of each study group is given below.

2.1.1 Arctotidinae (Arctotideae [Cichorioideae]: Asteraceae)

Arctotidinae is one of two subtribes recognized within the tribe Arctotideae (subtribes Gorteriinae, Arctotidinae) which is almost entirely restricted to Africa in its distributional range. The five genera (*Arctotheca* Vaill., *Arctotis* L., *Cymbonotus* Cass., *Dymondia* Compton, and *Haplocarpha* Less.) that make up the subtribe Arctotidinae comprise approximately 65 species, 44 of which are associated with the CFR. A recent biogeographic study made a first attempt at dating the diversification of the subtribe utilizing ITS mutation rates (McKenzie & Barker 2008). Based on these dates, the authors argued for a model of diversification in response to Miocene/Pliocene climatic change, as had been previously proposed for other Cape clades. The DNA matrix used in this study was obtained from McKenzie and Barker (2008) which includes duplicate accessions for a number of taxa. Duplicate accessions were trimmed after phylogeny reconstruction according to the following rules: (i) if duplicate accessions were reconstructed as 'sister' pairs, one of the two accessions was selected and removed; (ii) if duplicate accessions were reconstructed as 'paraphyletic', the two duplicates' positions were compared to McKenzie and Barker (2008) simplified cladogram (see Figure 4 in their study) and accessions trimmed accordingly. The phylogeny presented here contains approximately 65% of Cape taxa.

2.1.2 *Stoebe* clade (Gnaphalieae [Asteroideae]: Asteraceae)

As currently circumscribed, the *Stoebe* clade consists of five genera, *Amphiglossa* DC., *Bryomorphe* Harv., *Disparago* Gaertn., *Elytropappus* Cass., and *Stoebe* L. (Bergh 2009, PhD thesis). The clade is nested within the Gnaphalieae s.s. (Bayer *et al.* 2000; Bergh & Linder 2009) and encompasses 62 species that are centred in the Cape. Bergh (2009) presented the first DNA-based phylogenetic hypothesis for the *Stoebe* clade, which rendered an unresolved tree topology with a polytomy subtending the genera *Disparago*, *Elytropappus* and *Stoebe*. Whether or not this polytomy is the result of a recent rapid radiation event or a lack of data remains to be resolved. Here I make use of Bergh's data matrix (2009) (ETS + psbA-trnH) in combination with another nuclear gene region (ITS) that has been subsequently sequenced (data provided by N. Bergh). Taxon names are not indicated on the tree presented here because the phylogeny has not yet been published. The phylogeny presented contains approximately 96 % of Cape taxa.

2.1.3 Coryciinae (Diseae [Orchidoideae]: Orchidaceae)

The orchid sub-tribe Coryciinae comprises five genera (*Disperis* Sw., *Corycium* Sw., *Ceratandra* Eckl. Ex Bauer, *Pterygodium* Sw., and *Evotella* Kurzweil & H.P. Linder) that include approximately 120 species. Of these, 42 taxa occur in the CFR, and 27 are endemic to the CFR which inhabit a variety of soils and different altitudinal habitats (Goldblatt & Manning 2000). The five genera have previously been classed together on the basis of lip appendage morphology. Recent phylogenetic studies, however, showed that *Disperis* is isolated from the other four genera and, hence, that the subtribe Coryciinae s.s. is diphyletic (Waterman *et al.* 2009), which is compatible with earlier phylogenetic analyses of Orchidoideae. In addition, while *Disperis* and *Ceratandra* are monophyletic, *Corycium* and *Pterygodium* are paraphyletic. The DNA matrix obtained from Waterman *et al.* (2009) contains about 85% of the Cape taxa assigned to the subtribe Coryciinae, as well as a number of accessions at the sub-species level. Due to the paraphyly of this group, the *Disperis* and *Pterygodium* (*Pterygodium*+*Evotella*+*Ceratandra*) clades were analysed separately from each other subsequent to molecular dating of the Coryciinae clade.

2.1.4 *Satyrium* Sw. (Diseae [Orchidoideae]: Orchidaceae)

Of the 91 recognized species of *Satyrium* Sw., which is distributed throughout sub-Saharan Africa, Madagascar and Asia (Linder & Kurzweil 1999; van der Niet *et al.* 2005), 29 taxa are represented in the CFR and 18 are endemic to the CFR (Goldblatt & Manning 2002). Phylogenetic reconstruction of the genus has been problematic due to incongruence between nuclear and plastid markers (van der Niet & Linder 2008). DNA data obtained from the latter study contained 27 of the 29 Cape taxa. For the purpose of this work, I included only 14 of the Cape taxa which formed a well resolved clade (this group also included a subspecies of *S. stenopetalum*). Due to incongruence between gene regions, the method of Pirie *et al.* (2008) was adopted, whereby conflict taxa are decomposed into their conflicting gene accessions (nuclear versus plastid) prior to combined analysis. Preliminary analyses showed that this conflict was inconsequential in the case of *Satyrium* since conflict was restricted to non-Cape taxa, which were not incorporated in subsequent analyses that made use of the reconstructed tree topology.

2.1.5 *Pentameris* E. Mey. (Danthonieae [Danthonioideae]: Poaceae)

Pentameris E. Mey. is well-known and equally well-studied (Galley & Linder 2007; Galley *et al.* 2009), comprising 82 species that are distributed throughout sub-Saharan Africa. With 56 species native to the CFR, it is the most species-rich group of grasses in the region (Galley & Linder 2007). The group previously encompassed *Pentameris*, *Pentaschistis*, *Prionantium* plus *Pseudopentameris obtusifolia*, these taxa recently being unified under the name *Pentameris* s.l. (Linder *et al.* 2010). Most of the Cape species inhabit sandstone derived soils, while others are endemic to the more fertile shale-derived and calcareous substrates (Galley & Linder 2007). For molecular dating, the DNA data matrix obtained from Pirie *et al.* (2008) was trimmed to include the Cape genera *Merxmuellera*, *Capechloa*, *Geochloa*, *Tenaxia*, *Schismus*, *Tribolium* and *Pentameris*. Due to incongruence between nuclear and plastid gene regions, I adopted the same approach as for *Satyrium* (see 2.1.4), whereby gene incongruence was found in seven of the clade's Cape taxa. Separate state reconstruction analyses were then performed on trees in which conflict taxa were represented by their nuclear and plastid accessions. For the *Pentameris* clade, approximately 87% of Cape species were included in Pirie *et al.*'s DNA sequence matrix (2008).

2.1.6 *Ehrharta* Thunb. (Ehrharteae [Ehrhartioideae]: Poaceae)

The genus *Ehrharta* Thunb. is widespread in southern Africa, but has its centre of diversity in the CFR. Of the 23 African species, 20 occur in the CFR, and 12 are endemic to the region. The genus is represented by a variety of growth forms (suffrutescent, geophytic, caespitose and annual growth forms), making it morphologically highly diverse (Verboom *et al.* 2003). The group represents a classic example of an adaptive radiation in the Cape, likely in response to Miocence climatic changes (Verboom *et al.* 2004). Phylogeny reconstruction was based on the latest DNA sequence matrix obtained from Verboom *et al.* (2003) which sampled almost 90% of Cape taxa.

2.1.7 *Elegia/Thamnochortus* clade (Restionaceae)

The *Elegia/Thamnochortus* clade investigated here comprises *Elegia* L., *Askidiosperma* Steud., *Thamnochortus* P.J. Bergius, *Rhodocoma* Nees, as well as two *Restio* species (*R. egregius*, *R. micans*). With the exception of the latter two species, the four genera were analysed as a unit

based on evidence for their collective monophyly (Linder & Hardy 2010). The *Elegia* clade currently encompasses 48 species of *Elegia* and 12 species of *Askidiosperma*. The clade is a diverse and dominant element of the Cape flora, occurring in most habitats, though *Askidiosperma* occurs predominantly in mountainous regions in the western half of the CFR (Moline & Linder 2005, 2006; Linder & Hardy 2010). *Thamnochortus* comprises 32 species (Linder 1991, 2002; Hardy & Linder 2005; also Linder & Hardy 2010) that are largely restricted to the CFR (Goldblatt & Manning 2000). Since the genus is both morphologically and ecologically diverse, its diversification has been interpreted as dominated by ecological speciation, tightly coupled with habitat gradients (Hardy & Linder 2005). Comprising eight species, *Rhodocoma* is a small genus of African restioids that is distributed mainly across the eastern half of the CFR (Hardy & Linder 2007). Previous studies inferred a role for sympatric, ecological speciation in the diversification of *Rhodocoma* (Linder & Vlok 1991; Hardy & Linder 2007). The DNA data matrix was obtained from Linder & Hardy (2010) and trimmed to include only the above-mentioned genera (~90% species sampling), as well as two outgroup taxa (*Sporodanthus tasmanicus* and *Baloskion tetraphyllum*) required for calibration purposes.

2.1.8 *Tetraria* P. Beauv. (Schoeneae: Cyperaceae)

Tetraria P. Beauv., a genus of schoenoid sedges, is distributed across southern Africa, Australia and New Zealand. Phylogenetic studies have shown the South African lineages to be polyphyletic with two distinct clades (Verboom *et al.* 2006). Here, I focus on the reticulate-sheathed clade (*sensu* Slingsby & Verboom 2006) which is entirely restricted to the CFR and will refer to it as *Tetraria* throughout the thesis. Most recent work, though unpublished, has confirmed the monophyly of this clade and proposed a role for allopatric speciation across soil moisture and nutrient gradients in the diversification of the clade (Slingsby 2011). Phylogenetic and dating analyses were based on one nuclear (ETS) and four plastid gene regions (rps16, rbcL, psbA, trnLF; Table 2.1).The DNA sequence matrix, which contains 74% of Cape taxa, was obtained from Slingsby (2011).

2.1.9 *Protea* L. (Proteeae [Proteoideae II]: Proteaceae)

One of the characteristic genera of the Cape flora is *Protea* L., a genus comprising 112 species, 70 of which occur in the CFR and 69 being endemic to the region. While the genus has its centre of endemism and diversity in the Cape, a number of taxa are also found in the Drakensberg, and extend across the high-altitude habitats of the East-African Mountains as well as occurring in savanna woodlands and grasslands on the Kalahari plateau. Most recent work suggests that the high species diversity in this group in the Cape is the result of a gradual accumulation of species over time, rather than being the product of recent, rapid radiation (Valente *et al.* 2010). The DNA data matrix was obtained from Valente *et al.* (2010). Sampling for this group attained 98% of currently recognized Cape taxa.

2.1.10 *Leucadendron* R. Br. (Leucadendreae [Proteoideae II]: Proteaceae)

As another one of the charismatic genera of the CFR, *Leucadendron* R. Br. was selected here as a second representative of the Cape Proteaceae, despite being comparatively thinly sampled. The group comprises 85 currently recognized species and 11 subspecies, distributed almost entirely across the CFR (Goldblatt & Manning 2000; Barker et al. 2004) and occupying a variety of soil types. It is one of four dioecious genera in *Protea*ceae, with several lineages in the genus having evolved a variety of reproductive and survival strategies (Barker *et al.* 2004). DNA data were obtained from Barker *et al.* (2004) which included one gene region (ITS) sampled for approximately 73% of Cape taxa.

Table 2.1 List of the phylogenetic datasets included in this study. Density of sampling, calculated as a percentage of species listed for the CFR by Goldblatt and Manning (2002), was determined by the availability of both DNA accessions and georeferenceable data. Also given are the gene regions and substitution models for each, based on model selection by MrModeltest 2.3, and the respective source publications for each data set. Across all groups, only molecular data were used for phylogeny reconstruction and molecular dating (where morphological data were available, these were excluded from the analyses in order to standardize across all groups).

Family	Clade	Sampling	Markers & models selected			Source
			nuclear	Plastid	Model	
Asteraceae	Arctotidinae	**65%**	ITS		GTR+G	McKenzie & Barker 2008
				psbA-trnH	GTR+G	
				trnT-trnLF	GTR+G	
	Stoebe	**96%**	ITS		GTR+G	Bergh 2009
			ETS		HKY+I+G	
				trnLF	F81	
				trnTL	GTR	
				psbA-trnH	GTR+G	
Orchidaceae	*Disperis*	**83%**	ITS		GTR + I + Γ	Waterman *et al.* 2009
	Pterygodium	**83%**				
				matK	GTR + I + Γ	
				trnLF	GTR + Γ	
	Satyrium	**93%**	ITS		GTR + Γ	van der Niet & Linder 2008
				matK	GTR + I + Γ	
				trnLF	GTR + Γ	
				trnL	GTR + I	
				trnSG	GTR + Γ	
Poaceae	*Pentameris*	**87%**	ITS		GTR + I + Γ	Pirie *et al.* 2008
				trnLF	GTR + I + Γ	
				rpl16	GTR + I + Γ	
				atpB-rbcL	GTR + Γ	
	Ehrharta	**85%**	ITS		GTR + Γ	Verboom *et al.* 2003
				trnLF	GTR + Γ	
Restionaceae	*Elegia/ Thamnochortus*	**90%**		trnK-matK	GTR + I + Γ	Hardy *et al.* 2008
				atpB-rbcL	GTR + I + Γ	
				trnLF	GTR + I + Γ	
Cyperaceae	*Tetraria*	**74%**	ETS			Slingsby 2011
				rps16		
				rbcL		
				psbA		
				trnLF		
Proteaceae	*Leucadendron*	**73%**	ITS		GTR + I + Γ	Barker *et al.* 2004
	Protea	**98%**	ITS		GTR + I + Γ	Valente *et al.* 2010
				atpB-rbcL	HKY+G	
				rps16	GTR+G	
				trnLF	GTR + I + Γ	

2.2 Phylogenetic and molecular dating analyses

2.2.1 Choice of dating methods

The concept of the 'molecular clock' is based on the observation that most mutations in genes and proteins are effectively neutral and thereby accumulate at a relatively constant rate over time. These 'random ticks' are assumed to confer a more or less linear rate of molecular evolution (Pauling & Zuckerkandl 1965; Kimura 1986; Bromham & Penny 2003). Assuming the existence of such a clock, the divergence level between lineages reflects relative time since divergence, and can be calibrated using fossil records, palaeogeographical events, the age of strata to which taxa are endemic (Heads 2005; Renner 2005), or by using known external rates (Drummond *et al.* 2006; Ho 2007). Several different methods for estimating divergence dates are available, of which the most commonly used and most sophisticated include nonparametric rate smoothing (NPRS, Sanderson 1997), penalized likelihood (PL, Sanderson 2002), Multidivtime (Thorne & Kishino 2002) and Bayesian Evolutionary Analysis by Sampling Trees (BEAST, Drummond & Rambaut 2007).

Here, I have adopted a Bayesian dating approach as implemented in BEAST (v1.5.3), which employs a Bayesian Markov Monte Carlo Chain (MCMC) analysis to sample the tree and parameter space. BEAST has the advantage that the implementation of complex evolutionary models is relatively straightforward and that it incorporates, or at least addresses, some of the weaknesses of other molecular dating techniques. Primarily, unlike other dating techniques (e.g. Multidivtime), BEAST does not require a single 'input' tree topology on which the molecular clock is modelled. By simultaneously sampling for the optimum tree topology from the 'tree' landscape (posterior distribution) as well as incorporating priors on specified nodes to time-calibrate the branch lengths, BEAST is able to account for phylogenetic uncertainty.

In addition, BEAST accommodates non-constancy in rates of molecular evolution. A variety of factors influence the rate at which sequences evolve (i.e. the substitution rate); including generation time, metabolic rate, mutation rate and effective population size (Rutschmann 2006; Ho 2009). Thus, a constant substitution rate can rarely be validly assumed across all branches of a phylogenetic tree, rate heterogeneity being widespread and the norm rather than the

exception (Gillespie 1986; Renner 2005; Ho 2009). Although several dating methods address rate heterogeneity by applying a 'relaxed clock' model of evolution, most methods (PL, NPRS, Multidivtime) do so by assuming that lineages inherit their rates from their parent lineages ('rate autocorrelation'; Renner 2005; Rutschmann 2006; Pulquerio & Nichols 2007; Ho 2009). The general validity of this assumption is contested on the grounds that over very long timescales, the variation in organismal traits (life history, metabolic rate, generation time) that are assumed to underlie rate autocorrelation, becomes so large that the rate autocorrelation between lineages might be expected to break down (Drummond *et al.* 2006). BEAST avoids the problem of assuming rate autocorrelation through the application of an uncorrelated relaxed clock model (Drummond *et al.* 2006). In this model, the rate for each branch is sampled independently from an underlying log-normal or exponential rate distribution and there is no assumption that rates are autocorrelated.

Finally, BEAST allows calibration uncertainty (i.e. uncertainty in the age of fossils) to be integrated in a realistic and meaningful manner. While most other dating methods rely on 'point' estimates as calibration constraints, BEAST allows the user to specify a 'confidence distribution' as the prior for a given calibration (for a detailed review, see Ho & Philipps 2009). Hence, the uncertainty around the age of a fossil can be incorporated in the form of a pre-specified probability distribution, where the prior probability distribution is defined as a uniform, normal, lognormal, exponential or gamma distribution. While other molecular dating methods also allow for confidence intervals to be set around the calibration age, the confidence intervals are set by 'hard bounds' (i.e. Multidivtime). Such an assumption is arguably invalid given the uncertainty around most fossil age estimates (Pulquerio & Nichols 2007; Yang & Rannala 2006). In conclusion, BEAST incorporates both phylogenetic uncertainty as well as calibration age uncertainty. While this produces wider error margins around node age estimates, the resulting estimates are likely be more accurate and realistic (Ho & Philipps 2009).

2.2.2 Details of dating analyses

Instead of performing likelihood ratio tests to test the molecular clock hypothesis across all groups, as described by Huelsenbeck & Crandall (1997), the standard deviation of the uncorrelated lognormal relaxed clock (termed 'ucld.stdev') obtained from the BEAST output

serves as a measure as to whether the application of a relaxed clock is appropriate (Drummond *et al.* 2006; BEAST manual). This parameter gives an indication of 'clock-like' behaviour in a given data set. If the posterior distribution is centred about zero, the molecular clock hypothesis cannot be rejected. If, however, parameter estimates approach a value of 1, the molecular clock can be rejected and a relaxed clock is justified (Drummond *et al.* 2006; BEAST manual).

Branch lengths can be calibrated, by (i) applying independently-determined 'universal' substitution rates (Drummond *et al.* 2006; Ho 2007), (ii) applying secondary calibrations obtained from previously dated phylogenies, (iii) constraining nodes to ages based on palaeogeographic events, or by (iv) placing fossils of known ages directly on pertinent nodes (Heads 2005; Renner 2005). While direct fossil calibration is generally preferable, fossil evidence at the genus-level is often extremely scarce, as is the case for the clades sampled in this study (Linder 2003). Hence, a two-step approach was used to date these lineages. The first step involved calibrating a set of higher-level phylogenies, obtained from previous publications (listed in Table 2.2). In cases where detailed dating analyses existed, these were evaluated in terms of the placement and validity of the fossils, and subsequently re-run in BEAST, either omitting or including selected fossils (see Table 2.2). The second step entailed calibrating the species-level phylogenies using age estimates for specific nodes obtained from the fossil-calibrated higher-level phylogenies. Secondary calibrations, and results from these must, however, be both used and interpreted with caution (Shaul & Graur 2002).

For the higher-level phylogenies, node calibration followed the same protocol across all groups. For internal nodes, fossil calibrations were assigned to stem nodes, rather than to the crown nodes of the clade representing the synapomorphy of the fossil morphology. Fossils are generally assigned to clades whose extant members possess the synapomorphic features shown by that fossil. Based on this, fossil calibrations should be assigned to the crown node rather than to the stem node of the clade. Since the extinct lineage representing the fossil could be placed anywhere along the branch between the stem and crown node of the clade, however, placing the fossil on the stem node is a more conservative approach that is considered essential for accurate calibration and estimation of divergence dates (Renner 2005; Forest 2009). Since earliest known fossils indicate the minimum ages of lineages, priors on fossil-calibrated nodes

were set as lognormal (see Ho & Philipps 2009) with the (log) mean of the lognormal distribution set such that the median equalled the estimated minimum age of the fossil. This allows for the node to be slightly younger (accommodating error in fossil age), but considerably older (accommodating fossil age error and the possibility of older, as yet undiscovered fossils). Given that single fossils do not provide an indication on the form of the lognormal distribution, the (log) standard deviation was, by necessity, set arbitrarily to 0.5*(log) mean, such that the 95% CI spanned a realistic time period. The zero offset was set to the median minus 0.1*median. For most nodes, this gave very reasonable estimates. For younger nodes, however, subtracting only 10% of the median meant that the 95% CI were often too narrow to be realistic or to cover the full range of possible ages for the relevant fossil. Therefore, for nodes younger than 20 million years, 20% of the calibration value was subtracted from the median to give the zero offset.

For species-level phylogenies, the prior probability distribution on the calibration ages was set as a normal distribution, with a median and 95% CI set to match the mean and 95% CI of the posterior distributions obtained from the higher-level dating analyses.

Different substitution models were applied to individual gene regions, with model selection done under the Akaike Information Criterion (AIC) using MrModeltest v2.3 (Nylander 2004; Table 2.1). Xml files were assembled in BEAUTi v1.5.3 (Drummond & Rambaut 2007). Due to constraints on the higher-level phylogenies in the form of fossil calibrations, initial starting tree computation in BEAST failed for the higher-level analyses. For these, randomly resolved starting trees with imposed calibration constraints were provided in the xml files. In the case of species-level analyses, the initial starting tree was computed in BEAST using a tree prior defined by a Yule process.

For the higher-level studies, three analyses with randomly resolved starting trees (with calibration nodes constrained) were run separately for 5×10^6 generations until convergence was reached, sampling every 5,000 generations (except in the case of Asteraceae, which was run for 10^7 generations). Species-level analyses were also run for 5×10^7 generations, sampling every 5,000 generations. Effective Sample Size (ESS) values were assessed using Tracer v1.5 (Rambaut & Drummond 2007). In each case, the burn-in was set to 10%, i.e. the first 1,000 trees (or 5×10^6

generations) were discarded for Maximum Clade Credibility (MCC) tree estimation using TreeAnnotator v1.5.3 (Rambaut & Drummond 2007). In all cases, this ensured that only the stationary distribution was sampled. These MCC trees, and trimmed versions thereof, were used for subsequent analyses (ancestral state reconstructions, phylogenetic independent contrasts).

Table 2.2 List of higher-level phylogenetic datasets that were used for estimating secondary calibration points (for the species-level phylogenies), the gene regions used in each dataset and each dataset's source publication. Nodes are labelled as in Figure 3.1 - 3.3. The BEP clade comprises Bambusoideae, Ehrhartoideae, Pooideae, and the PACCAD clade comprises Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, Danthonioideae (GPWG 2001). Asterisk (*) denotes a fossil that was originally attached to Mutisieae (Zavada & deVilliers 2000) but is actually Dicomeae, see Torices (2010).

Family (Source)	Node	Age (Ma)	Fossil	Description	Reference
Asteraceae	A	≥ 23	*Artemisia*-type pollen	Stem node of Anthemideae	Graham 1996
Panero &	B	≥ 25	*Ambrosia*-type fossil	Stem node of Heliantheae s.l.	Graham 1996
Funk 2008	C	≥ 38	*Dicoma*-type fossil*	Stem node of Dicomeae	Scott *et al.* 2006
	D	≥ 34	*Mutisiapollis patersonii*	Stem node of Mutisieae	Martinez-Millan 2010; Barreda *et al.* 2010
	E	≥ 47	Fossil capitulum	Split Barnadesioideae – Mutisioideae s.l. + Carduoideae	Barreda *et al.* 2010b
Orchidaceae	A	15-20	(*Meliorchis caribea*	Stem of Goodyerinae	Ramirez *et al.* 2007
Ramirez *et al.*	B	20-23	*Dendrobium winikaphyllum*	Split *Dendrobium - Bulbosa*	Conran *et al.* 2009
2007	C	20-23	*Earina fouldenensis*	Split *Earina - Agrostophyllum*	Conran *et al.* 2009
Poales	A	≥ 60	Cyperaceae fruit	Stem node of Cyperaceae	See Bremer 2002
Christin *et al.*	B	≥ 70	*Milfordia* pollen	Stem node of Restionaceae	Linder 1987
2008	C	≥ 70	*Monoporites* pollen	Stem node of Poaceae	Linder 1987; Herendeen & Crane 1995
	D	≥ 55	Multiflowered grass spikelet	Stem node of BEP-PACCAD	Crepet & Feldman 1991; GPWG 2001
	E	≥ 35	*Dicanthelium* phytolith	Split BEP-PACCAD	Strömberg 2005
	F	≥ 19	*Setaria* anthecia	Stem node of Chloridoideae	Elias 1942
	G	≥ 40	Mapanioid fossil sedge	Stem node of Mapanioideae	Smith *et al.* 2009
Proteaceae Sauquet *et al.* 2009				Analysis not repeated.	

2.2.3 Selection of fossil calibrations

Attempts to date Asteraceae have been confounded by a paucity of fossils. The most detailed review on the asteraceous fossil record is given by Graham (1996), while a more recent review provides a detailed summary of the basal Asteraceae fossils, as well as those of Menyanthaceae, Goodeniaceae and Calyceraceae (Barreda *et al.* 2010a). The two 'supertree' analyses that have been performed to date (Funk *et al.* 2005; Torices 2010), in which dates are ascribed to specific parts of a phylogeny, do not yield a robust set of date estimates and are, instead, suitable only for providing relative branch lengths for comparative purposes (Torices 2010). Here, I made use of a trimmed version (73 accessions of the original 108) of Panero & Funk's (2008) DNA sequence matrix for Asteraceae, in combination with a selection of fossils described in the literature for estimating divergence time (Table 2.2). Due to the unavailability of matching gene regions, no extra taxa were added. Instead, one or more accessions were included in the two asteraceous species-level phylogenies (Arctotidinae, *Stoebe*) in order to obtain overlapping nodes for the purpose of calibration.

In contrast to the orchid family's exceptional diversity across the globe, its fossil record is poor, with few fossils being unequivocally assignable to specific nodes of the Orchidaceae phylogeny (Ramirez *et al.* 2007). Few dating studies have consequently emerged for the family and the family's time of origin is contentious (Ramirez *et al.* 2007). The recent discovery and description of a preserved orchid pollinarium (*Meliorchis caribea*) yielded the first detailed divergence time estimation of Orchidaceae using penalized likelihood and NPRS (Ramirez *et al.* 2007). Gustafsson *et al.* (2010) subsequently published a robust dating analysis of the orchid family using the above-mentioned orchid pollinarium, as well as two recently described orchid fossils, *Dendrobium* and *Earina* (Conran *et al.* 2009). Here, I made use of the 61-taxon DNA matrix obtained from Ramirez *et al.* (2007) and the same three fossil calibrations as used by Gustafsson *et al.* (2010). In contrast to Gustafsson *et al.* (2010), I assigned fossils to stem nodes as opposed to crown nodes. In addition, 23 accessions were added to permit calibration of species-level phylogenies (see Appendix Table A.1).

Table 2.3 Prior and posterior distributions of secondary calibration points for the species-level phylogenies obtained from the dated higher-level phylogenetic data sets. HL Node refers to the respective node in the higher-level dataset (and is labelled accordingly in the HL phylogeny, see Figure 3.1 – 3.3), SL Node depicts the respective node in the species-level data set. Posteriors depict the mean and 95% confidence intervals for each node as obtained from the higher-level dating analyses, and modified as 'Priors' for the species-level dating analyses. Asterisk (*) marks the two clades *Protea* and *Leucadendron*, for which the numbers given in brackets behind the node description refer to the node numbers as in Sauquet *et al.* 2010.

Group	Node	Description	Posterior (UCLN)		Prior (UCLN)		
			Mean	95% CI	Mean	SD	95% CI
Asteraceae							
Arctotidinae	AA	Crown Cichorioideae	31.46	[23.32-38.90]	31.43	3.9	[23.36-38.64]
Stoebe	AS	Crown of Gnaphalieae	18.99	[11.56-25.78]	19.02	3.0	[13.12-24.88]
Orchidaceae							
Coryciinae	OC1	Split Codonorchis – Coryciinae/Orchideae/Disinae	42.19	[32.48-52.70]	42.41	5.1	[32.41-52.41]
	OC2	Split Disperis - Pterygodium	34.53	[26.53-44.45]	34.92	4.4	[26.29-43.54]
Satyrium	OS1	Split Disperis - Satyrium	34.53	[26.53-44.45]	34.92	4.4	[26.29-43.54]
	OS2	Split Orchideae - *Satyrium*	23.12	[16.22-30.82]	23.45	3.5	[16.59-30.31]
Poaceae							
Danthonioideae (Pentameris)	PP1	Split Chloridoideae – Danthonioideae	23.73	[19.02-28.87]	24.17	2.7	[18.93-29.40]
	PP1	Stem Pentameris	17.47	[12.99-23.14	17.51	2.6	[12.41-22.60]
Ehrharta	PE	Split Bambusoideae + Pooideae – Ehrhartoideae	33.55	[28.49-39.73]	34.08	2.9	[28.30-39.86]
Restionaceae							
Elegia/ Thamnochortus	PET1	Crown *Baloskion*	27.45	[14.37-43.83]	28.51	7.3	[14.20-42.82]
Cyperaceae							
Tetraria	PT	Split *Hypolytrum* + *Paramapania* – Cyperaceae	48.15	[39.09-58.20]	48.43	4.6	[39.41-15.73]
Proteaceae*							
Protea	PP1	Crown Proteoideae II (B)	75.66	[71.36-80.20]	75.66	2.2	[71.25-80.07]
	PP2	Crown Proteeae (67)	30.25	[16.75-46.38]	30.25	7.6	[15.35-45.15]
	PP3	Crown Leucadendrinae (59)	28.11	[18.57-38.27]	28.11	5.1	[18.11-38.11]
	PP4	Crown *Protea* (66)	12.32	[5.06-20.56]	12.32	3.9	[4.56-20.08]
Leucadendron	PL1	Crown Leucadendreae (61)	44.52	[31.96-58.26]	44.52	6.8	[31.17-57.87]
	PL2	Crown Adenanthos+Leucadendrinae (60)	33.91	[22.84-45.59]	33.91	5.9	[22.19-45.63]
	PL3	Crown Leucadendrinae (59)	28.11	[18.57-38.27]	28.11	5.1	[18.11-38.11]
	PL4	Crown *Serruria* (54)	16.35	[10.23-22.37]	16.35	3.1	[10.27-22.43]

The Poales are globally widespread in the world and have received considerable attention in the literature. Fossil pollen, spikelets and flowers are relatively abundant owing to the group's cosmopolitan distribution and abundance throughout angiosperm history. There have been several attempts to date this group, or at least some of its subgroups such as Poaceae (Bremer 2002; Vicentini *et al.* 2008; Christin *et al.* 2008; Bouchenak-Khelladi *et al.* 2009, 2010). Here, the DNA sequence matrix for Poales obtained from Christin *et al.* (2008) was calibrated with a selection of fossils that had been used in previous dating analyses (Vicentini *et al.* 2008; Bremer 2002) in addition to a recently described fossil (mapanioid sedge; Smith *et al.* 2009). The original DNA matrix, which consisted of 338 accessions, was trimmed to 90 accessions to facilitate computation. In order to date species-level phylogenies using secondary calibrations from relevant nodes on the higher-level phylogeny, 15 accessions were added to the trimmed DNA matrix (see Appendix Table A.1.).

Only in the case of Proteaceae (Sauquet *et al.* 2009) was the dating analysis not repeated, since fossil placement and dating methodology (BEAST) were comparable to the approach adopted here. Secondary calibration points for *Protea* and *Leucadendron* were directly extracted from the published chronogram (Sauquet *et al.* 2009, Supplementary Information Table S2).

2.3 Selection and scoring of habitat variables

In order to reconstruct ancestral habitat endemism and test whether subsequent habitat shifts occurred in response to climatic/geological changes in the CFR since the Early Miocene, the following habitat variables were selected for study: (i) rainfall seasonality (coefficient of variation of annual precipitation), (ii) mean annual precipitation (MAP), (iii) substrate, and (iv) vegetation type (Table 2.4). Both MAP and seasonality were selected as indicators of climatic change, since one is a measure of the volume of rainfall received per annum (MAP), while the other is a measure of the distribution of rainfall over the course of the year (seasonality).

Scoring of study species for each of the habitat variables was done within a GIS framework using different data sources for the different environmental variables (see Table 2.4). Since the aim of reconstructing habitat variables was to trace the switch from one state to another, continuous

variables (MAP, seasonality) were categorized into discrete ranges. The scoring of species for discretized continuous variables was based on the interquartile range (25-75%) of the continuous variable that was occupied by each taxon, rather than on the mean or median values, since these do not represent the range of habitats occupied by a particular species (Hardy & Linder 2005; Hardy 2006). The interquartile range (25-75%) was preferred over the min-max range as the former excludes outliers, thereby accounting for specimen misidentifications, incorrect localization of records and accommodating the imperfect resolution of the GIS layers used. In cases where the interquartile range of a particular taxon extended across two or more categories, the taxon was classified as being polymorphic for that particular environmental variable. In the case of discrete environmental variables (substrate, vegetation), an 80%-rule was applied for habitat scoring, equivalent to the 25-75%-rule applied to continuous variable scoring. Where more than 80% of records for a taxon occurred on one substrate type, the taxon was classified as occurring solely on that particular substrate. Where less than 80% of records occur in a specific substrate, the taxon was classified as 'polymorphic' for substrate preference. Due to the computational constraints of the selected ancestral state reconstruction method on the number of different states that can be reconstructed, categorization of each habitat variable was limited to a maximum of six states.

To characterize the environments occupied by each species, herbarium records were acquired from the Bolus (BOL), Pretoria (PRE) and Compton herbaria (NBG), and georeferenced as accurately as possible, to a specific point locality within the CFR. Localities that could not be confidently assigned geographic coordinates due to missing or incomplete locality descriptions were omitted. Duplicate collection records were also omitted. In order to account for the variable degree of accuracy of locality descriptions, each georeferenced record was assigned a 'confidence level' of accuracy as follows: A = < 500m, B = 500m – 2km, C = 2km – 5km, D = 5km – 10km, E > 10km, F = GPS coordinates given (recorded at site of collection). Although it is preferable to use only the most accurately georeferenced records (i.e.: accuracy level A or F), this is not feasible since very few records would then be useable. Therefore, records with an accuracy level B were also included (i.e. all specimens with accuracy < 2000m). Since the accuracy-level classification used for *Stoebe* was slightly different (0 – 300m, 300 – 1000m, 1000 – 4000m, 4 – 10km, > 10km), all specimens with an accuracy of < 4000m were used for data

extraction. After this aforementioned data cleaning process was completed, the number of records used for habitat definition varied from 1 to 250 records per taxon. For each georeferenced record, the underlying environmental value was extracted in ArcGIS v9.3 using Hawth's Analysis Tools (Beyer 2004).

Finally, in order to assess the validity of this GIS-based classification approach for substrate scoring, GIS-based scoring results for six selected groups were compared against 'expert-based' scoring. The latter was based on information obtained from experts on a given group (G.A. Verboom, H.P. Linder, N. Bergh), herbarium records or from other sources (Protea Atlas; Restionaceae IntKey).

Table 2.4 Brief description and classification scheme for the four environmental variables that were used here to classify habitat endemism for each taxon, and the data source for each respective GIS-layer.

Variable/Class	Description	Resolution	Source
Seasonality (%)		~ 1km	WorldClim,
< 60	Aseasonal		Hijmans *et al.* 2005
> 60	Seasonal		
Mean annual precipitation (mm/a)		~ 1km	WorldClim,
< 300	Arid		Hijmans *et al.* 2005
300 - 599	semi-arid		
600 - 899	Mesic		
> 900	Wet		
Substrate		1:250,000	Lithology maps,
Quartzite	Sandstones, arenites of the Table Mountain, Witteberg Groups		Geoscience Council
Shale	Shale, mudrock of Bokkeveld, Cederberg, Pakhuis Formations		South Africa
Granite	Granites of Cape Granite Suite		
Alluvial	Alluvial deposits across mountains, along rivers		
Lowsands	Clays, sandy soils especially along the western coastal plain		
Calcareous	Calcretes, calc-arenites. Mostly along the coastal margin.		
Vegetation		1:250,000	Mucina & Rutherford
Fynbos	Heathlike vegetation (Proteaceae, Restionaceae, Ericaceae)		2006
Renosterveld	Heathlike vegetation *('*renosterbos'; Asteraceae, Iridaceae)		
Strandveld	Coastal scrub		
Succulent Karoo	Dwarf, succulent shrubs (Mesembryanthemaceae, Crassulaceae)		

2.3.1 Rainfall seasonality

Rainfall seasonality was scored using the coefficient of variation of monthly precipitation using the WorldClim Global Climate data (Hijmans *et al.* 2005; Bioclim variable 15) which have a

resolution of 30 arc-seconds. Here, this variable is given as a percentage of monthly variation in precipitation over the year (i.e. a high value represents a highly seasonal climate, while a low value represents a more aseasonal climate). Following Chase and Meadows (2007) the boundary between aseasonal and seasonal climate was defined by a seasonality score of 60%, with scores falling below the cut-off being defined as aseasonal, and scores over 60% as seasonal (Figure 2.2). Setting the boundary at 60% resulted in all of the eastern areas as well as the high-altitude zones in the west being classified as aseasonal.

2.3.2 Mean annual precipitation

Scoring of MAP was also based on the BioClim variables derived from the WorldClim Global Climate data (Hijmans *et al.* 2005). MAP (Bioclim variable 12) was arbitrarily classified into four categories representing the full range of MAP conditions in the CFR: < 300 mm/year (arid), 300-600 mm/year (semi-arid), 601-900 mm/year (mesic), and > 900 mm/year (wet) (Figure 2.3).

2.3.3 Substrate type

Substrates were classified using the 1:250,000 lithology maps of South Africa (Council for Geoscience) which classify the geology of the Cape into approximately 130 different lithologies. Six broad substrate categories were chosen to represent the diversity of substrates in the Cape. These are quartzitic sandstone, shale, granite, calcareous substrates, alluvial deposits and lowland sands (Figure 2.4; Table 2.4). One or more substrate classes were assigned to each taxon, depending on which substrate(s) it predominantly inhabits.

2.3.4 Vegetation type

Vegetation types were classified on the basis of the 1:250,000 vegetation maps provided by Mucina and Rutherford (2006). The four vegetation types used to classify the vegetation of the CFR were fynbos, renosterveld and strandveld and succulent karoo (Figure 2.5).

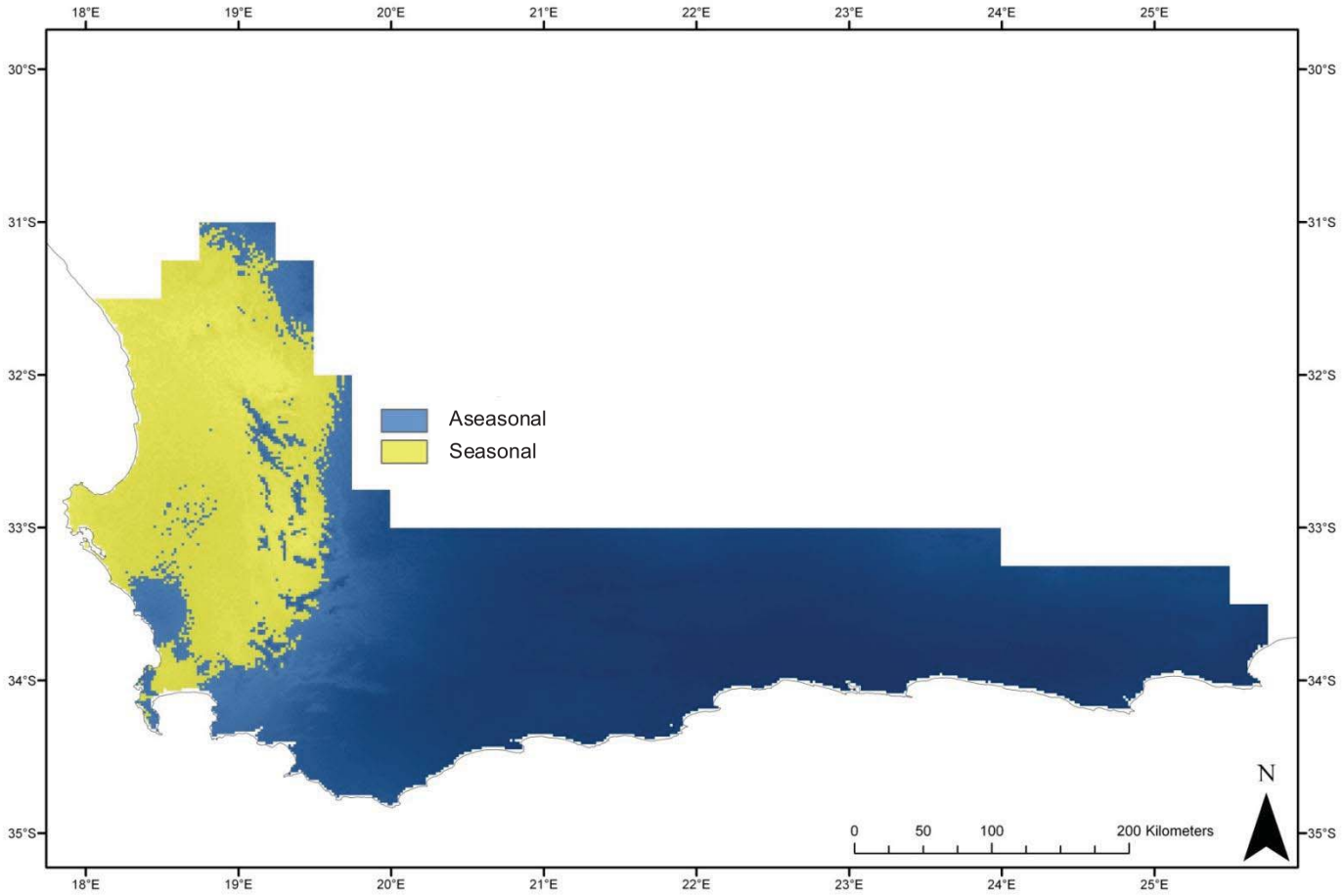

Figure 2.2 The precipitation seasonality index, depicted as the coefficient of variation of monthly precipitation across the CFR. Data was derived from the WorldClim dataset (Bioclim variable 15; Hijmans *et al.* 2005). Aseasonal (blue) environments are defined as those where the CV is less than 60%, i.e. rain falls throughout the year. Seasonal (yellow) environments are those where the CV is greater than 60%, and in the CFR, this means rain falls predominantly over the winter months.

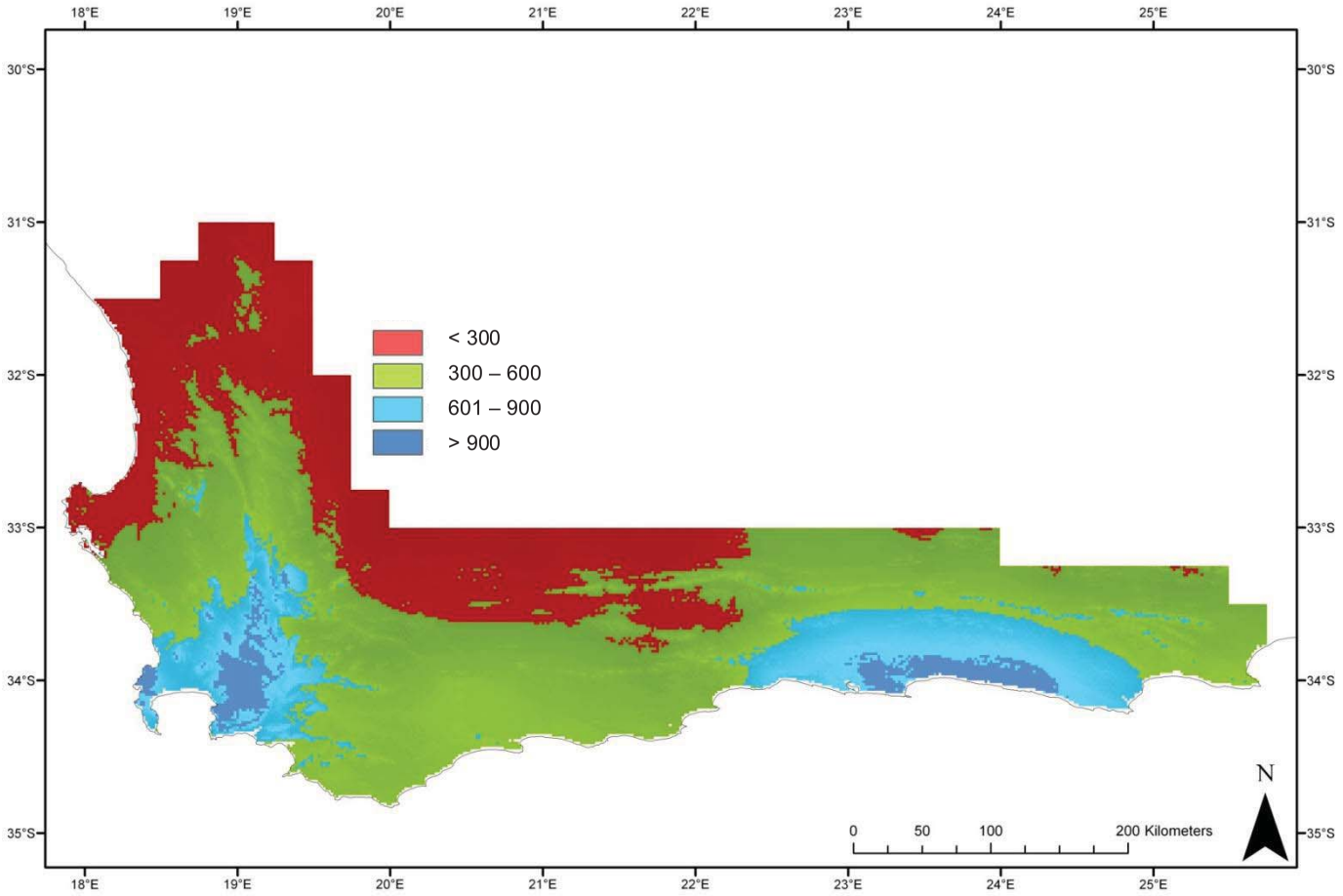

Figure 2.3 Mean annual precipitation (MAP) across the CFR. Data was derived from the WorldClim dataset (Bioclim variable 12; Hijmans *et al.* 2005). MAP categories were arbitrarily assigned.

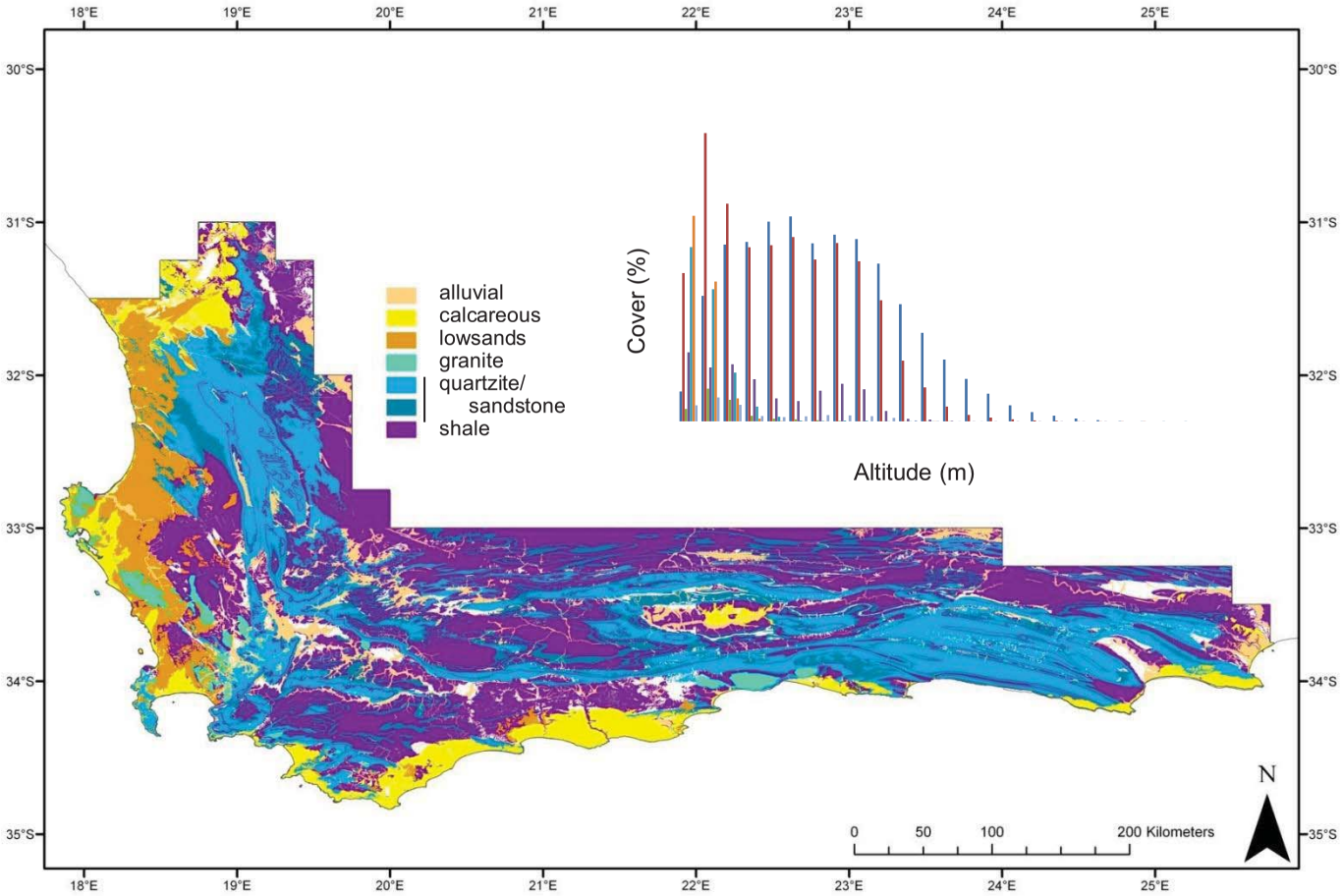

Figure 2.4 The six major substrate types that are represented in the Cape Floristic Region (sandstone, shale, calcareous soils, granite, alluvial deposits and lowland sands). The map was modified from the 1:250,000 lithology map obtained from the Geoscience Council, South Africa. Inset graph shows the percentage cover of each substrate at different altitudes.

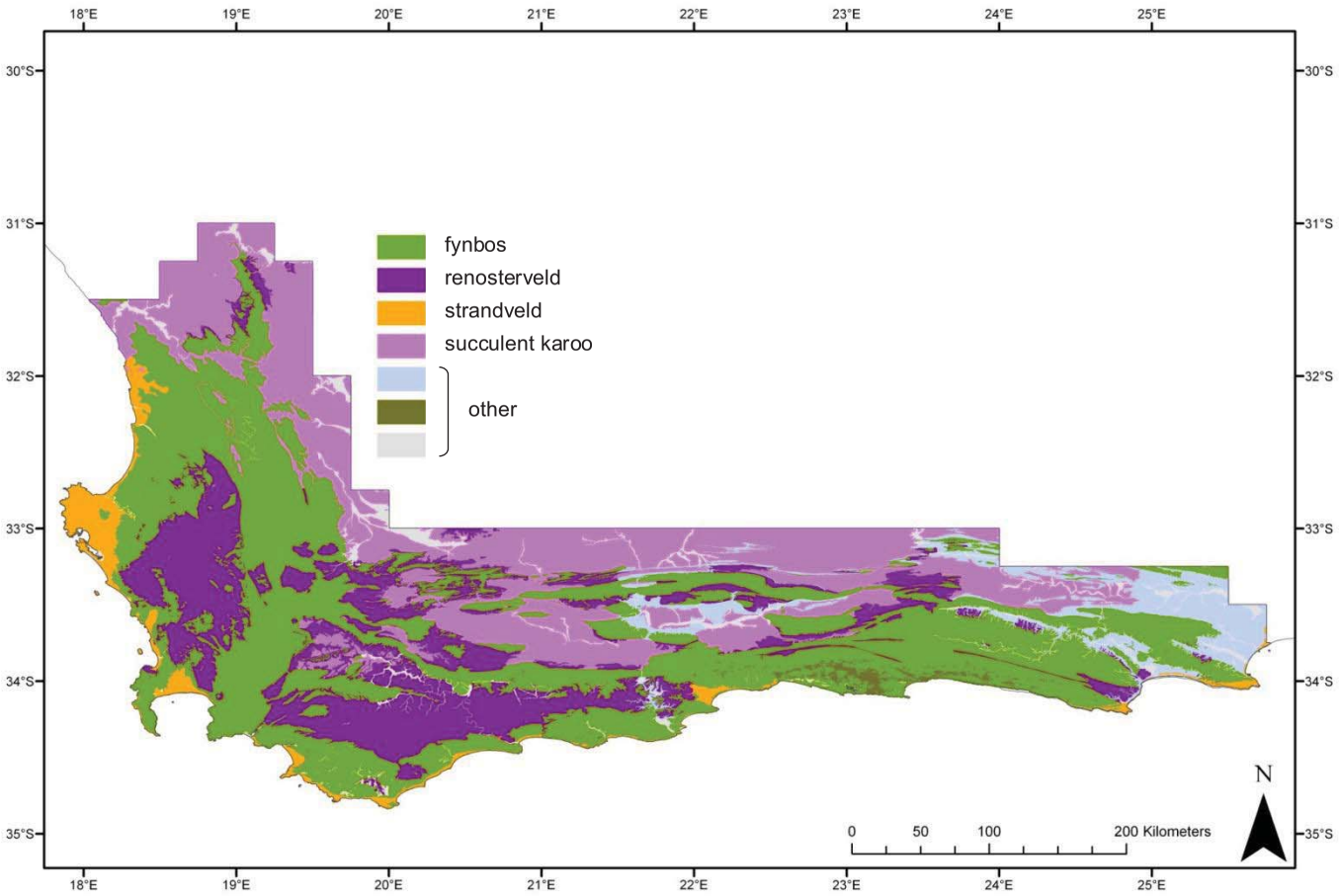

Figure 2.5 The major vegetation types represented in the CFR. The map was derived and modified from Mucina and Rutherford (2006). In this study, focus is only on the fynbos, renosterveld, strandveld and succulent karoo vegetation, which cover the majority of the region.

2.4 Ancestral State Reconstructions

Ancestral character state reconstruction (ASR), or character optimization, is a popular and powerful method for gaining insight into the evolutionary history of lineages in relation to both character evolution and historical biogeography (Cunningham *et al.* 1998; Cunningham 1999; Losos 1999; Swofford & Maddison 1992). A variety of different optimization methods are available, including parsimony (Swofford & Maddison 1992), maximum likelihood (Schluter *et al.* 1997; Pagel 1999), stochastic character mapping (Huelsenbeck *et al.* 2003) and Bayesian inference (Pagel *et al.* 2004). Each of these has its own set of constraints and assumptions. As highlighted in recent reviews and studies using ancestral state reconstructions, the choice of analytical method, and the nature of the variable being reconstructed greatly affects the outcome of ASR, and remains a highly contentious issue in evolutionary and comparative biology (Losos 1999; Ekman *et al.* 2008; Omland 1999; Cunningham 1999; Xiang & Thomas 2008).

2.4.1 Choice of reconstruction methods

A recurring theme concerning the choice of ASR methods is the importance of incorporating both phylogenetic and mapping uncertainty, as well as the accommodation of state polymorphisms at ancestral nodes (Hardy & Linder 2005; Hardy 2006). Only a full Bayesian approach, as implemented in BayesMultistate (Pagel *et al.* 2004), takes into account both phylogenetic and mapping uncertainty. This method, however, fails to accommodate polymorphism of the ancestral character states, which is, in fact, a considerable limitation of most ASR methods (Hardy & Linder 2005; Hardy 2006). Polymorphism is the norm rather than the exception for most habitat/ecological variables (Hardy 2006) and it seems unrealistic and impractical to impose monomorphic states on polymorphic ancestral nodes. Since this thesis is concerned principally with reconstructing ancestral habitats, I opted to make use of a method which allows for polymorphism at the ancestral node but does so at the expense of accommodating phylogenetic uncertainty. While parsimony optimization with polymorphism coding would be suitable for this, it fails to incorporate branch length information (Hardy & Linder 2005). Therefore, I employed a method which utilizes a continuous-time model (the dispersal-extinction-cladogenesis, DEC) developed specifically for geographic range evolution

(Ree & Smith 2008; Lamm & Redelings 2009), but which is also appropriate for ancestral habitat inference. Phylogenetic uncertainty is accommodated here to a certain degree since this method incorporates branch length information from the BEAST MCC input trees required for state reconstruction (i.e. uncertain branches are typically short owing to the lack of character change between lineages). The model is implemented within a maximum likelihood framework in LagRange (Ree & Smith 2008). Besides allowing polymorphism to be reconstructed at ancestral nodes, one of the main advantages of this technique is the option of including transition matrices that allow the user to define biologically meaningful priors on the reconstruction of polymorphism of ancestral nodes (Lamm & Redelings 2009; Buerki *et al.* 2010). These matrices allow the user to impose constraints on the transitions between character states, as well as assigning prior probabilities to specific transitions across pre-defined time periods (Range and Dispersal constraints, respectively). Thus, it is possible, for example, to order states.

2.4.2 Details of ancestral state reconstruction analysis

Input files were assembled using the online LagRange configurator. In the case of substrate and vegetation type, the transition matrix was defined without any constraints on the transitions between different habitat types. However, habitat transition matrices were 'ordered' for the categorized continuous variable MAP. Since seasonality was a binary character, it was not ordered. The dispersal matrix for all five variables was set to a probability of 1.0 across all transitions, and the time period was defined from zero to the age of the root node + 1 million years (the earliest period has to be set to be older than the root node of the given phylogeny). Rate parameters such as baseline rates of dispersal and extinction were set to be estimated. Reconstructions were done using trimmed versions of MCC trees obtained from the BEAST dating analyses. Outgroup taxa, taxa that do not occur in the Cape, and taxa for which no georeferenced locality data) was available, were pruned from the MCC tree prior to optimization analyses.

Habitat shifts were defined here as transitions from an ancestrally either polymorphic or monomorphic state to a derived state that is significantly different from the ancestral state. Based on the LagRange output, which provides probability estimates of each state for each

branch, 'monomorphic' branches were defined as those for which the probability of the ancestral lineage occupying a particular state was greater than 60%. Ideally, this threshold should be 90 - to 100%. Given the error that is associated with steps prior to ancestral state reconstruction (i.e. error in georeferencing), a lower threshold of 60% was chosen to accommodate the error margin. I will focus both on shifts that involve only strictly 'endemic' lineages, i.e. from a monomorphic/polymorphic ancestor to a monomorphic descendent, as well as shifts that involve a monomorphic ancestor and a polymorphic descendent.

In order to test whether the emergence of a seasonal flora was associated with upwelling-induced aridification during the Mid Miocene (10 – 14 Ma) or with uplift-induced aridification during the Miocene-Pliocene (5 Ma) a t-test was used to calculate whether the first appearance of seasonal endemics was significantly different from these proposed dates. Similarly, whether the emergence of the shale- and calcrete flora was associated with Pliocene tectonic uplift (3 – 5 Ma), a t-test was used to calculate whether the first appearance of shale- and calcrete-endemics was significantly different from the proposed dates. In addition, matched-sample t-tests were used to evaluate whether the onset of seasonality coincided with the shift to shale or calcrete, and whether the latter two shifts occurred at the same time. Statistical tests were run in R v2.13.2 (R Development Core Team 2011).

2.5 Diversification patterns and processes

In order to compare the rate of lineage accumulation across the selected clades and to test the hypothesis that climatic and/or tectonic events triggered the radiation of selected Cape floral clades, the rate of lineage accumulation was quantified for each clade. The net diversification rate (r) is a product of both speciation (λ) and extinction (μ) rates in a given lineage ($r = \lambda - \mu$) (Magallon & Sanderson 2001). One of the confounding factors in estimating net diversification rates, then, is the challenge of estimating extinction rates, given that phylogenetic trees only represent extant lineages.

Here, net diversification rates were estimated for each 'Cape' clade using the 'rate estimate' method of Magallon and Sanderson (2001). This algorithm fits a constant-rate birth-death

model to the data given a relative extinction rate (ε = μ/λ), thereby estimating a relative rate of cladogenesis over time in a given clade. Rate estimates were calculated for each group based on their respective crown group ages, both under a model of no extinction (ε = 0.0) and relatively high extinction (ε = 0.9).

Tests for rate shifts in diversification rate were performed using the delta-AICrc test (Nee *et al.* 1994; Rabosky 2006a, b). This method fits rate-constant and rate-variable birth-death (or pure birth) models to a given MCC tree, the best-fit model then being selected by evaluating alternative model fits under the Akaike Information Criterion (AIC). Rate-variable models include the exponential and linear density-dependent models (DDX and DDL, respectively), as well as a two-rate yule model (yule2rate). This method was preferred over the constant rates test (γ-statistic; Pybus & Harvey 2000), which only identifies pulses of rapid rate change that involve a rapid decline in the rate of lineage accumulation, yet fails to detect rapid rate increases (Rabosky 2006a).

Lineage-through-time (LTT) plots were generated for each of the 'Cape' clades in order to depict lineage accumulation graphically, and to compare clade age and lineage accumulation rates between the different groups. Despite their usefulness in depicting lineage accumulation, LTT plots must be interpreted with caution as they do not incorporate extinction. LTT plots and rate estimates were calculated using the packages *ape* (Paradis *et al.* 2004) and *laser* (Rabosky 2006b), respectively, in R v2.13.2 (R Development Core Team 2011). Time- and diversity-dependent models of diversification were fitted using the fitdAICrc function of the package *laser* (Rabosky 2006b) in R v2.13.2 (R Development Core Team 2011).

2.6 Morphological character divergence

To test the hypothesis that lineage diversification in the Cape was driven by different adaptive processes in the aseasonal (ancestral) versus seasonal ('novel') habitats, I adopted the following approach. Assuming that the level of phenotypic divergence is greater between lineages which have diverged under an adaptive radiation scenario than between lineages which have radiated non-adaptively, the relative importance of adaptive versus non-adaptive processes can be

explored at the phenotype-environment interface. This was done by regressing the absolute differences in the values of selected morphological traits at each node between sister lineages against the reconstructed seasonality score for each node describing this sister relationship.

Morphological divergence analyses were performed for four groups (*Ehrharta*, *Leucadendron*, *Protea* and *Tetraria*). Traits studied included leaf length and width, plant height and, depending on availability, a measure of the size of a reproductive structure (spikelet length, flower size, cone size). The choice of these traits was dependent on the availability of trait data in floras or other sources (i.e. directly from the authors, as in the case of *Tetraria*). At each node, the absolute amount of divergence in morphology between daughter lineages was calculated as the unstandardized phylogenetically independent contrast (PIC; Felsenstein 1985; see also McPeek 1995) at that node. PIC were calculated using the pic function in the *ape* library (Paradis *et al.* 2004) in R v2.13.2 (R Development Core Team 2011), and based on the MCC trees obtained from BEAST.

Since LagRange only reconstructs discrete characters, the seasonality score for each node was reconstructed using linear parsimony. The major advantage of linear parsimony in comparison to squared-change parsimony is that it does not have a tendency to spread character change throughout the tree, thereby 'centralizing' the ancestral state (Butler & Losos 1997; Losos 1999). In contrast, in linear parsimony character change is allowed to be 'concentrated' around specific nodes on the tree, following a more 'punctuated' model of evolution. Since the aim of this analysis is to detect shifts into novel environments, using linear parsimony is then a more appropriate model. For MinMax linear parsimony reconstructions, I used the upper and lower bounds of the interquartile range as minimum and maximum values (25% and 75%), respectively, in order to reduce the effect of outliers. Linear parsimony reconstructions were performed in R v2.13.2 (R Development Core Team 2011).

Ecological evolutionary processes (i.e. adaptive/non-adaptive) are often difficult to describe using general linear regression models, since these models assume equal variance in the underlying distribution of the data, and hence infer a single slope or rate of change for the relationship between variables (Cade *et al.* 1999; Cade & Noon 2003). Yet such processes are usually complex, being made up of a number of factors, not all of which are easily measured and

incorporated in statistical models. As a consequence, the distribution of ecological data typically has unequal variance. An important implication of such heterogeneous variation is that the relationship between the response and predictor variables is best described by multiple slopes. Therefore, I employed a quantile regression model which, unlike conventional linear regression models, estimates a range of rate changes along the variable's response curve (Cade & Noon 2003). Since I was only interested in the nature of the upper bound, i.e. what limits phenotypic differentiation, the quantile regression was based only on data points in the upper 0.75 quantile. Significance tests for regression analyses were performed using the asymptotic rank score tests (ARST) as implemented in Blossom statistical software (Cade & Richards 2005).

CHAPTER 3: RESULTS

3.1 Phylogenetic and molecular dating analyses

High effective sample size values (ESS > 200) indicated sufficiently long run-times of the MCC chains and adequate sampling of the parameter space for valid parameter estimation after the set generation time was reached for all 13 dating analyses (10^7 and $5x10^6$ generations for the higher-level, and $5x10^6$ generations for the species-level analyses). Based on the standard deviation of the UCLN relaxed clock, a strict molecular clock was rejected for all groups, justifying the use of a relaxed clock model (Table 3.1). Furthermore, the covariance parameter, which acts as a measure of rate autocorrelation, justified the use of a relaxed clock model across all dating analyses (Table 3.1). Where covariance values vary around zero, fast and slow rates are indicated to occur on neighbouring branches, providing no evidence for rate autocorrelation.

3.1.1 Topological comparisons

Maximum Clade Credibility (MCC) trees for Asteraceae, Orchidaceae and Poales obtained from BEAST were robustly supported and largely congruent with topologies generated by previous analyses (Figures 3.1 – 3.3). In Orchidaceae there was a minor topological inconsistency compared with the MCC tree published by Gustafsson *et al.* (2010). Where Gustafsson *et al.* (2010) reconstructed Cypridoideae as sister to Vanillioideae + Epidendroideae + Orchidoideae, this study resolved the relationship of these four groups as Vanillioideae being sister to Cypridoideae + Epidendroideae + Orchidoideae. In both cases, however, the contradicting node had poor support (PP < 0.70). The topology obtained here was consistent with the topology provided on the Angiosperm Phylogeny website (Stevens 2001).

The MCC trees for the 10 species-level data sets were robustly supported and generally congruent with previously published phylogenetic reconstructions (Figures 3.4 - 3.13). Most cases of incongruence between previously published and current tree topologies were minor, being confined to younger nodes which were generally poorly supported in both published and

current topologies (PP < 0.7; i.e. Arctotidinae, *Satyrium*, *Pentameris*, *Protea* and *Leucadendron*). There was only one case of significant topological incongruence. In the *Elegia/Thamnochortus* clade, *Askidiosperma* was reconstructed as sister to *Thamnochortus* + *Rhodocoma*, where it had previously been reconstructed as sister to *Elegia* (Hardy & Linder 2005). In this study, the node describing the split between *Elegia* and *Askidiosperma* + *Rhodocoma* + *Thamnochortus* was, however, well supported in the MCC tree (posterior probability, PP = 1.0; Figure 3.10).

Table 3.1 Summary table of the BEAST output file giving (i) the standard deviation of the UCLD clock rate, and (ii) the covariance which is a measure of autocorrelation. Given also are the respective 95% confidence intervals (CI).

Group	UCLD		Covariance	
	stdev (σ)	95% CI	mean	95% CI
Asteraceae	0.8869	0.7495 – 1.0231	0.1198	-0.0405 – 0.2759
Orchidaceae	0.4787	0.3906 – 0.5714	0.1057	-0.044 – 0.2534
Poales	0.6489	0.5597 – 0.7503	0.0762	-0.0452 – 0.2054
Arctotidinae	0.623	0.3925 – 0.8579	0.0384	-0.1075 – 0.1946
Stoebe	0.5773	0.3588 – 0.7969	0.0474	-0.149 – 0.2427
Coryciinae	0.4195	0.3389 – 0.501	0.0049	-0.1483 – 0.1494
Satyrium	0.6814	0.5534 – 0.8127	0.0639	-0.0631 – 0.189
Danthonioideae	0.6975	0.5964 – 0.8055	0.0754	-0.0282 – 0.1954
Ehrharta	0.6714	0.2982 – 1.0874	0.0308	-0.2327 – 0.3124
Elegia/Thamnochortus	0.3645	0.2582 – 0.4712	0.0071	-0.1296 – 0.143
Tetraria	0.4792	0.3369 – 0.6145	0.0593	-0.1144 – 0.2352
Protea	0.9003	0.7477 – 1.071	0.0928	-0.0467 – 0.2347
Leucadendron	0.8496	0.5505 – 1.1794	0.0398	-0.1409 – 0.2316

Similar to previously published reconstructions, there was conflict between nuclear and plastid accessions for a number of taxa in the *Satyrium* and *Pentameris* clades (Figure 3.7 and 3.8, respectively). This incongruence was addressed by splitting conflict taxa into their nuclear and plastid counterparts and allowing these to be resolved separately following the approach of Pirie *et al.* (2008). For both *Pentameris* and *Satyrium*, phylogenetic reconstruction then rendered the same tree topology as in previous reconstructions (Pirie *et al.* 2008; van der Niet & Linder 2008). In the case of *Satyrium*, conflict between nuclear and plastid accessions did not affect any of the Cape taxa; hence this did not affect any of the downstream analyses done here.

In contrast, seven Cape taxa were affected by gene incongruence in *Pentameris*, which impacted the outcome of subsequent ancestral state reconstruction (discussed later).

3.1.2 Date estimate comparisons

Crown node ages obtained here for the major families in the higher-level dating analyses (Asteraceae and Orchidaceae; Cyperaceae, Restionaceae and Poaceae as part of Poales) ranged from the Late Cretaceous to as early as the Oligocene (Figures 3.1 – 3.3), and were both older and younger than previously published date estimates (see Table 3.2 for a summary of age comparisons). With an estimated crown node age of 58.04 Ma, the origin of Asteraceae was dated here as being much older than suggested by a previous dating analysis using NPRS (~40 Ma; Kim *et al.* 2005). The latter study did not, however, include any reference fossils from Asteraceae, relying instead on an outgroup fossil (*Cornus*, Cornaceae). In contrast to the situation for Asteraceae, the estimated crown node age for Orchidaceae attained here (74.98 Ma) was broadly consistent with the crown node age estimate obtained by Gustafsson *et al.* (2010; 77.01 Ma), the same dataset and dating method (BEAST) being used to derive both date estimates. Within Poales, crown node ages for the three major families, Cyperaceae, Restionaceae and Poaceae, were dated to 48.34, 28.12 and 55.83 Ma, respectively. While the date for Cyperaceae is roughly consistent with that previously published by Christin *et al.* (2008; ~46 Ma), the crown node ages for Restionaceae and Poaceae were substantially younger than those previously obtained.

In the species-level data sets, the estimated crown node ages for various study groups varied from the Early to Late Miocene (Figures 3.4 to 3.13). The two oldest 'Cape' groups, *Protea* and *Leucadendron*, date back to the very early Miocene, having had their origins in the Cape at the latest 21.12 Ma and 21.17 Ma ago, respectively. The youngest group (*Stoebe*) originated in the Late Miocene, with an estimated crown node age of 7.09 Ma, while the origins of the remaining six groups in the Cape were estimated to fall between 10 and 20 Ma.

Comparisons of selected node ages obtained in this study with those from previously published dating analyses revealed considerable differences in age estimates (Table 3.2). Only in three cases were published age estimates similar to those obtained here (Orchidaceae, *Tetraria*,

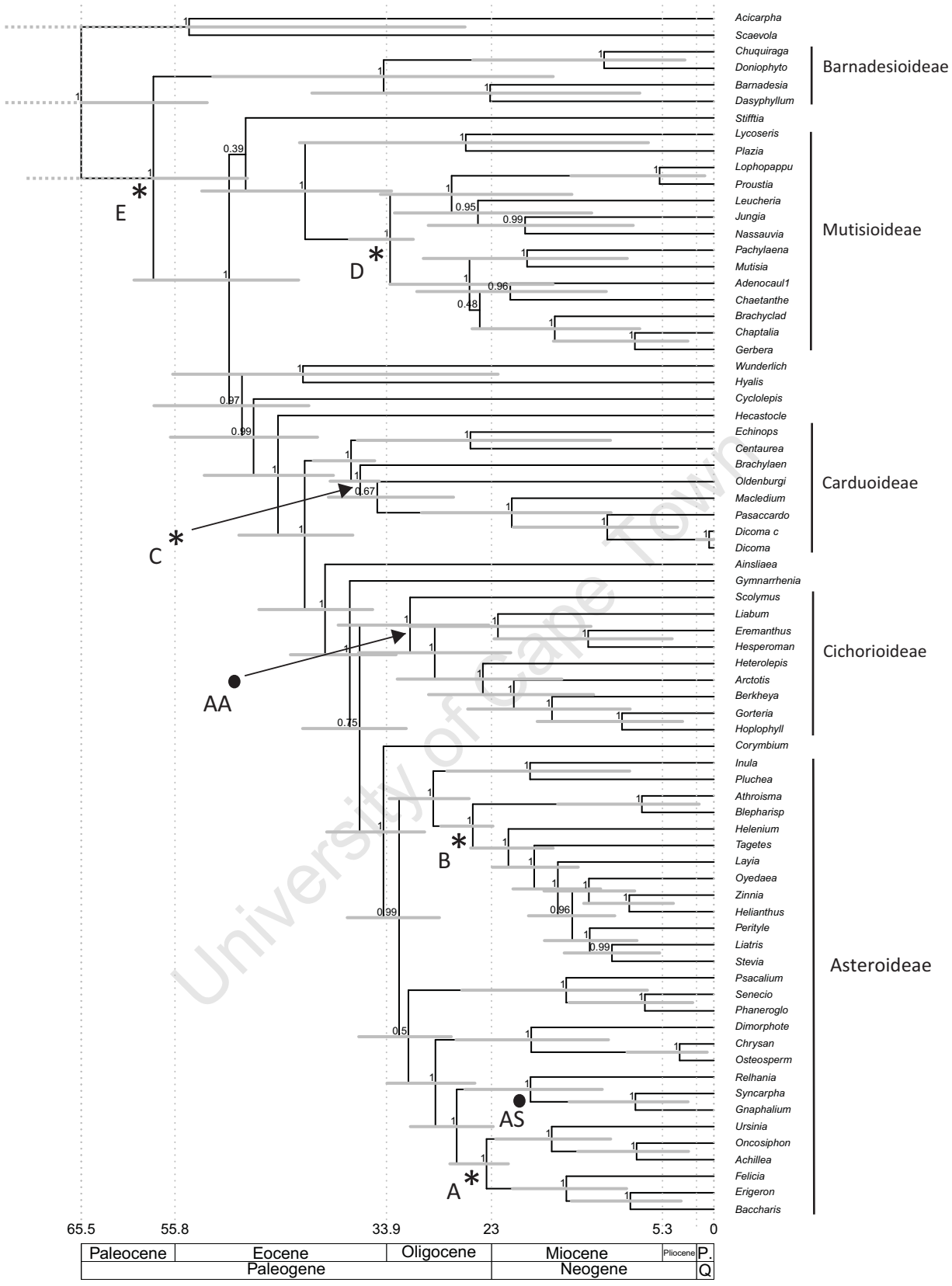

Figure 3.1 Dated Bayesian Maximum clade credibility tree for Asteraceae. Ages on nodes represent the median node height with bars indicating the 95% credibility interval. Calibration nodes are indicated by capital letters A - E (marked as stars, see Table 2.2 for detailed information on calibration ages). AA and AS depict nodes used as secondary calibration points for species-level dating analyses (filled circles: AA - Arctotidinae, AS - *Stoebe*).

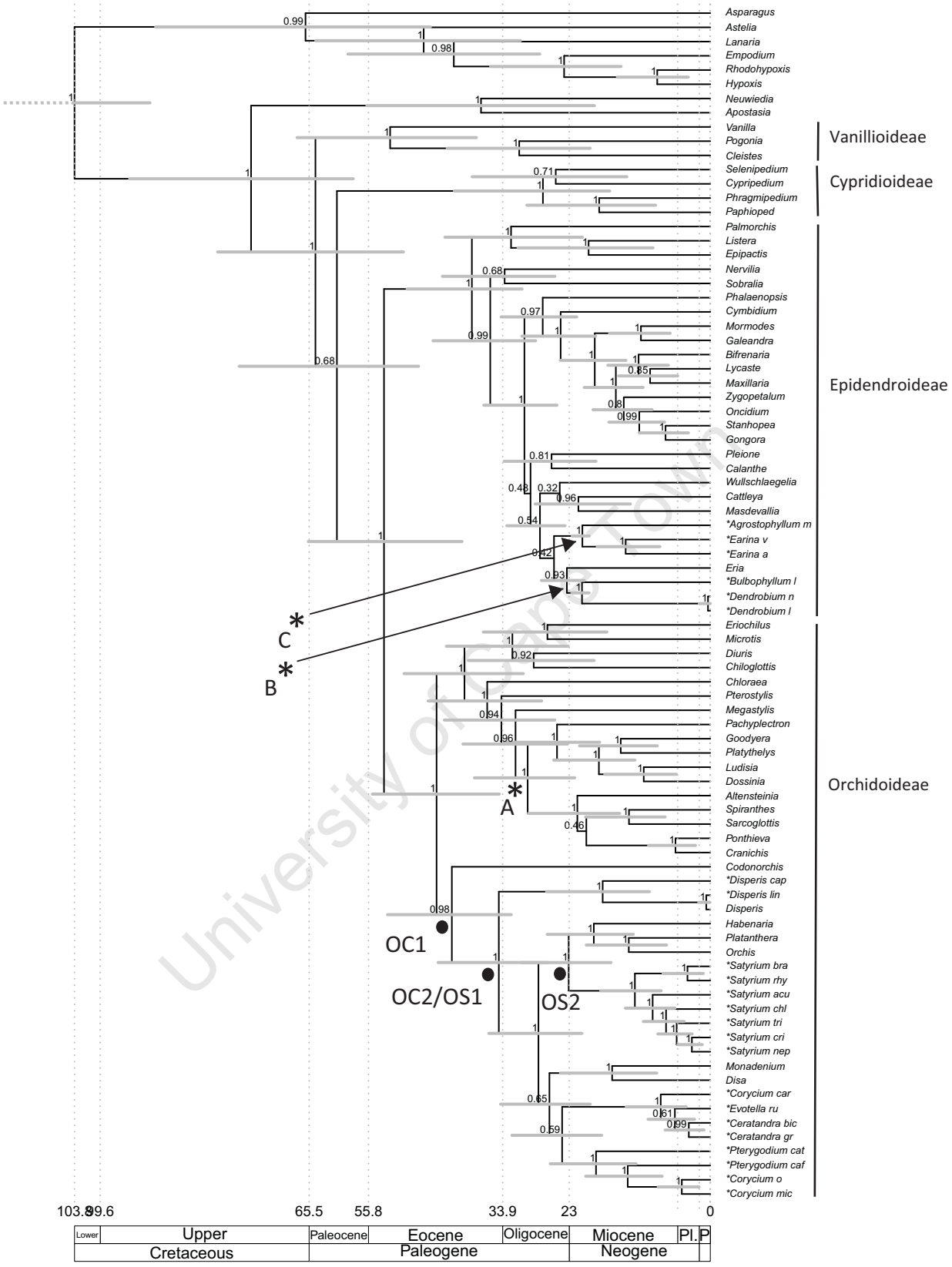

Figure 3.2 Dated Bayesian Maximum clade credibility tree for Orchidaceae. Ages on nodes represent the median node height with bars indicating the 95% credibility interval. Calibration nodes are indicated by capital letters A - C (marked as stars, see Table 2.2 for detailed information on calibration ages). OC1, OC2, OS1 and OS2 depict nodes used as secondary calibration points for species-level dating analyses (filled circles: OC - Coryciinae, OS - *Satyrium*). Taxa denoted with an asterisk (*) were added in order to obtain secondary calibration dates for specis-level phylogenies.

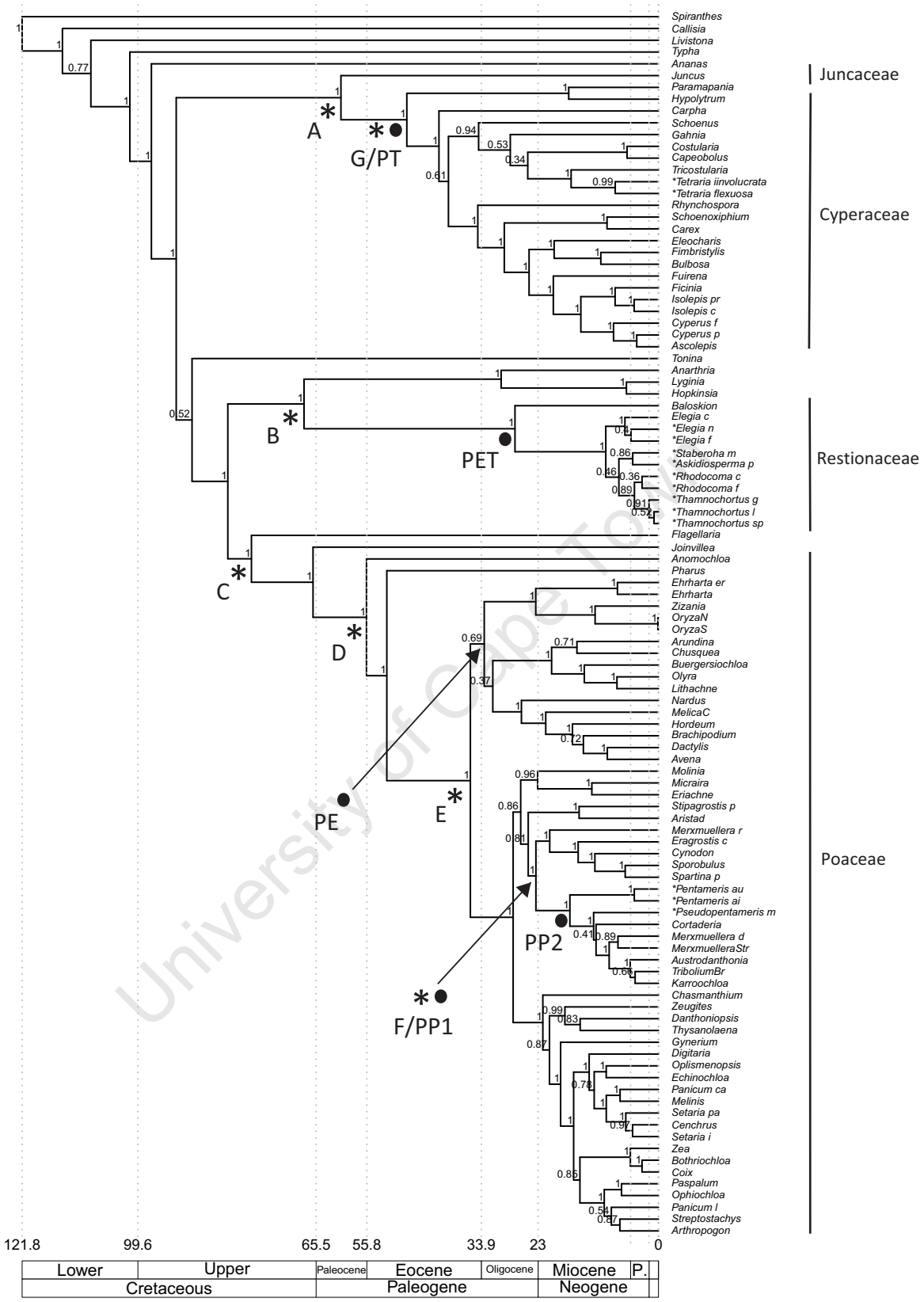

Figure 3.3 Dated Bayesian Maximum clade credibility tree for Poales. Ages on nodes represent the median node height with bars indicating the 95% credibility interval. Calibration nodes are indicated by capital letters A - G (marked as stars, see Table 2.2 for detailed information on calibration ages). PP1, PP2, PE, PET1, PET2, PT depict nodes used as secondary calibration points for species-level dating analyses (filled circles: PP - *Pentameris,* PE - *Ehrharta*, PET - *Elegia/Thamnochortus*, PT - *Tetraria*). Taxa denoted with an asterisk (*) were added in order to obtain secondary calibration dates for specis-level phylogenies.

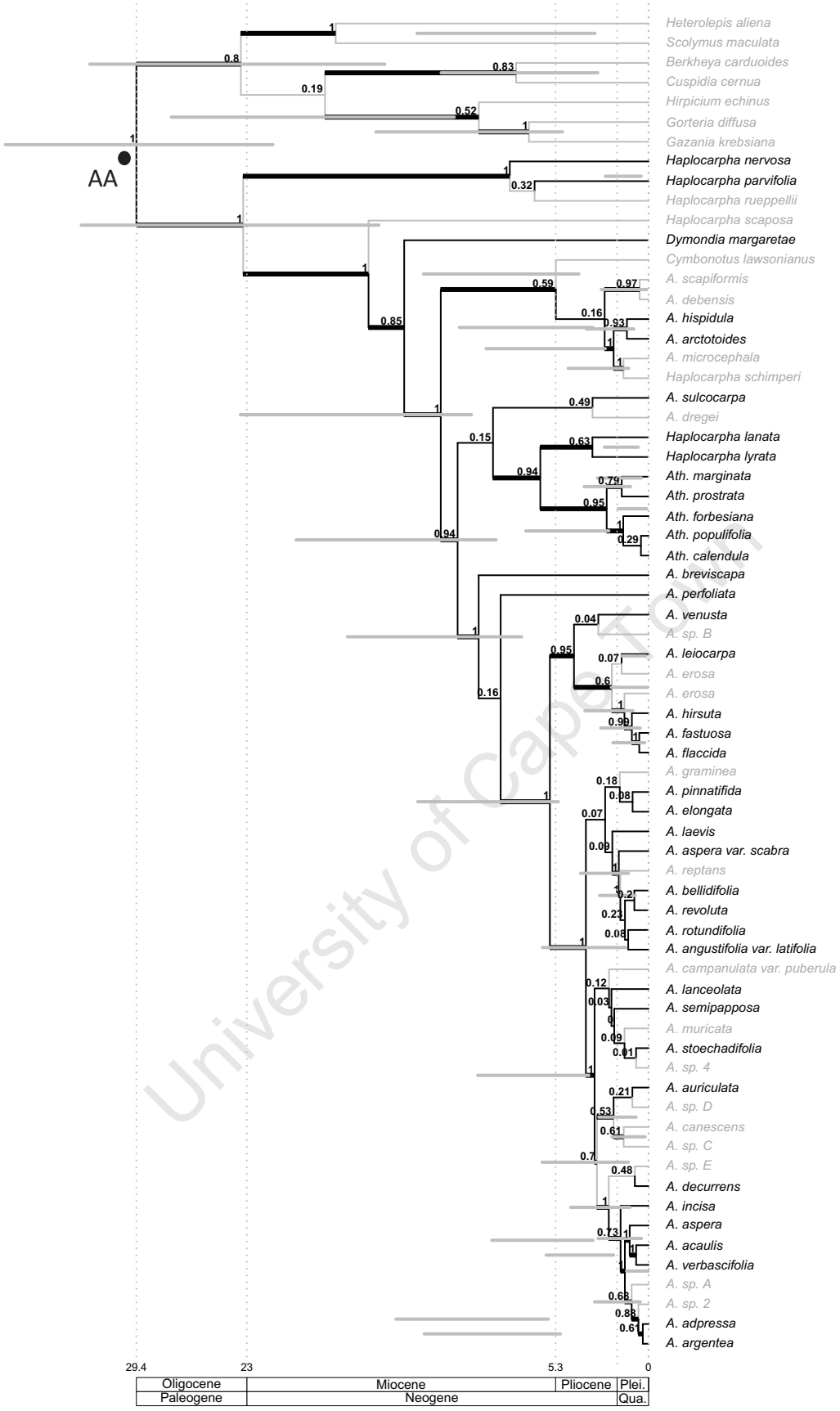

Figure 3.4 Dated Bayesian Maximum clade credibility tree for Arctotidinae. Numbers on nodes represent posterior probability support values. The calibration node is indicated by `AA' as in the higher-level phylogeny (Asteraceae) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

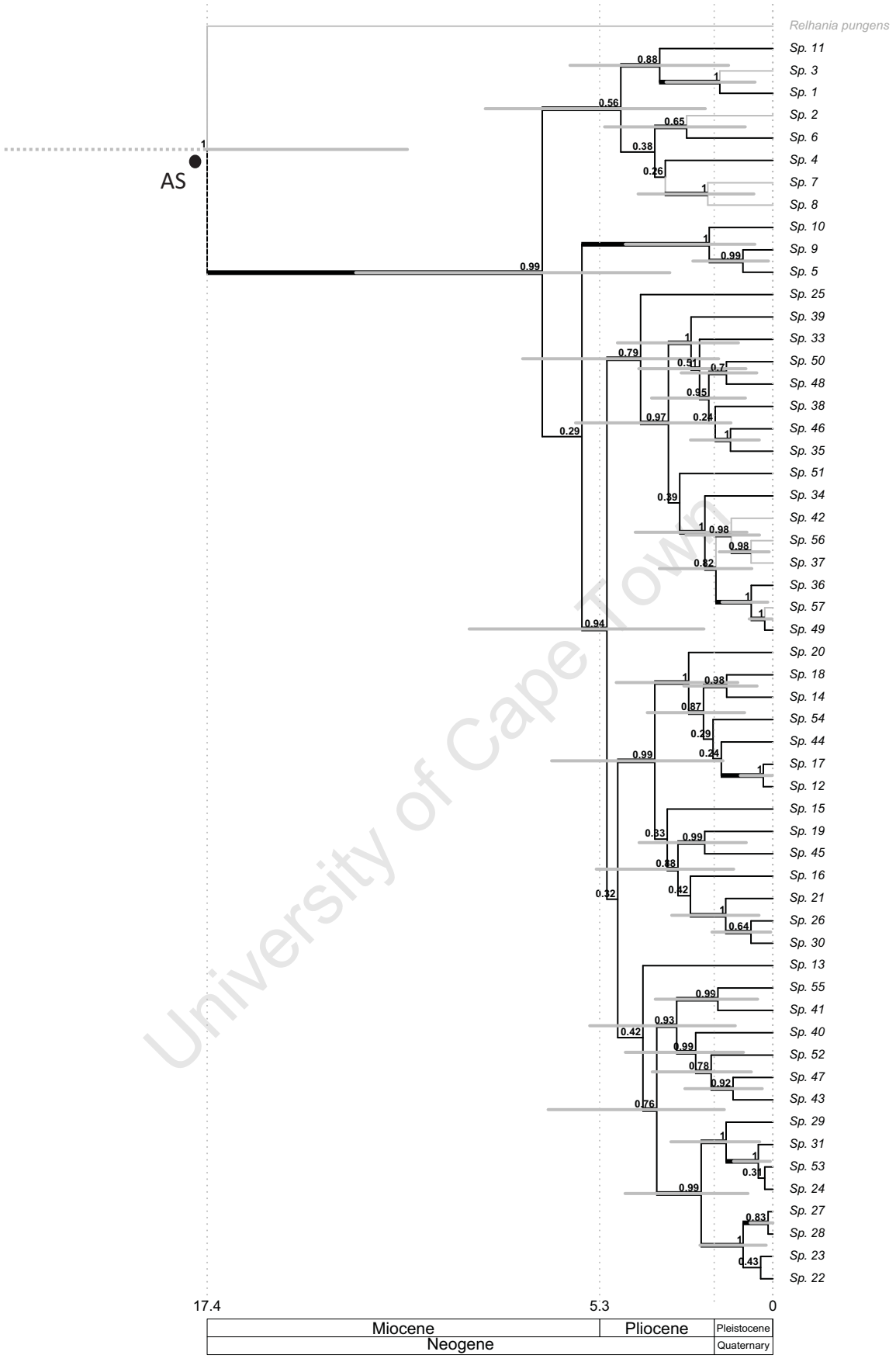

Figure 3.5 Dated Bayesian Maximum clade credibility tree for *Stoebe*. Numbers on nodes represent posterior probability support values. The calibration node is indicated by `AS' as in the higher-level phylogeny (Asteraceae) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region. Taxon names are not given since this is an unpublished data set.

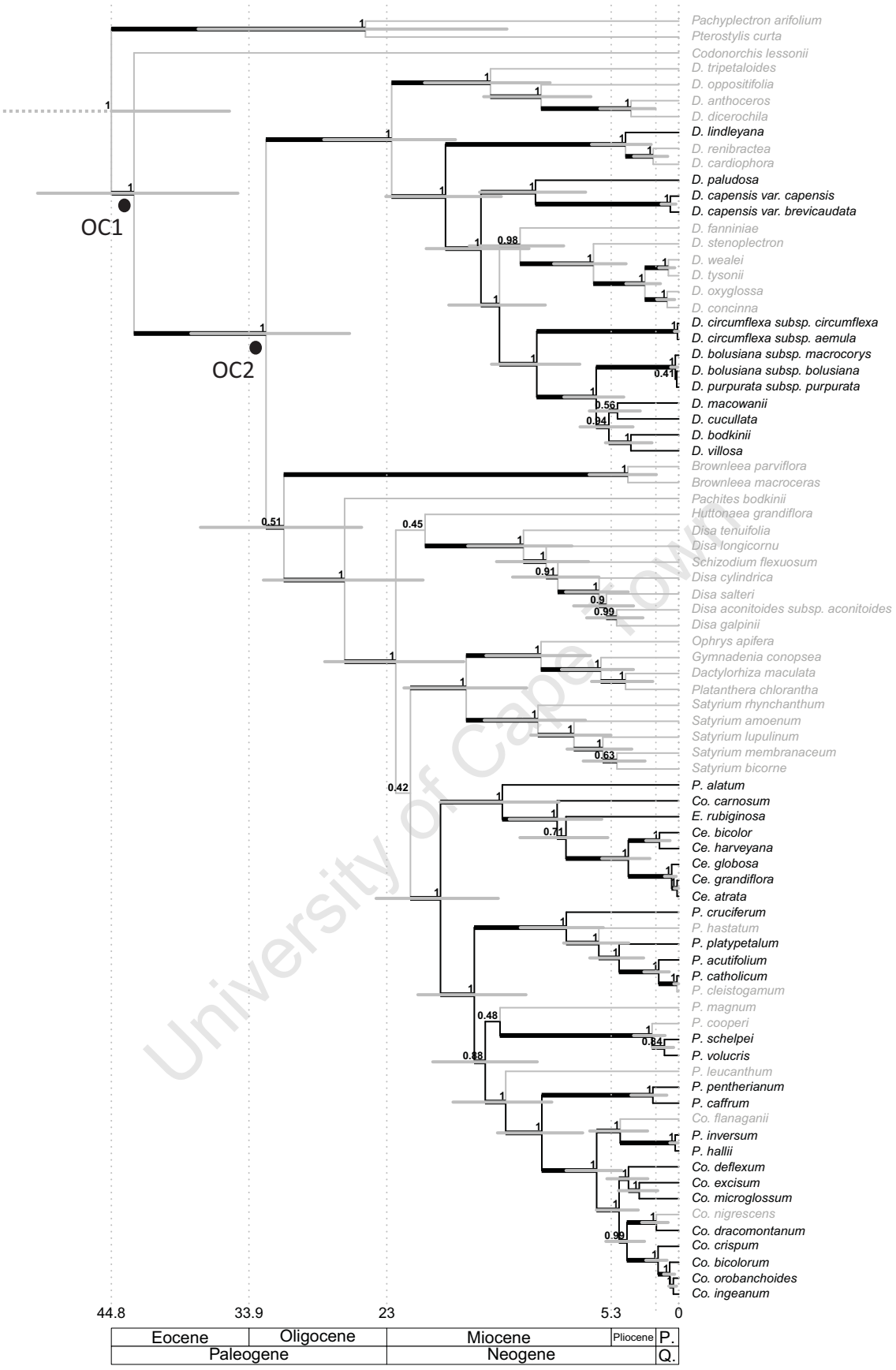

Figure 3.6 Dated Bayesian Maximum clade credibility tree for Coryciinae (*Disperis* and *Pterygodium*). Numbers on nodes represent posterior probability support values. Calibration node are indicated by `OC1' and `OC2' as in the higher-level phylogeny (Orchidaceae) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

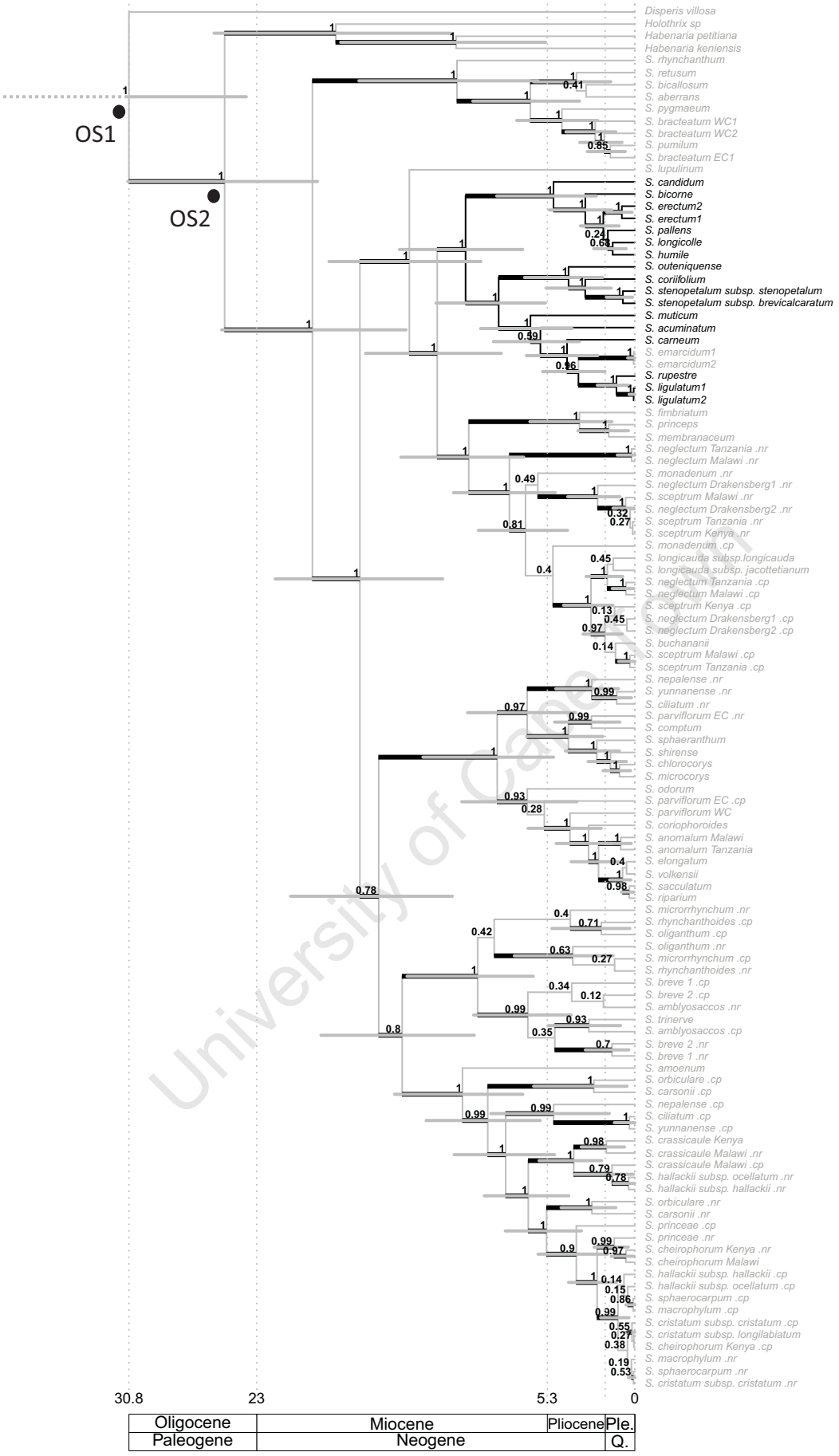

Figure 3.7 Dated Bayesian Maximum clade credibility tree for *Satyrium*. Numbers on nodes represent posterior probability support values. Calibration node are indicated by `OS1' and `OS2' as in the higher-level phylogeny (Orchidaceae) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

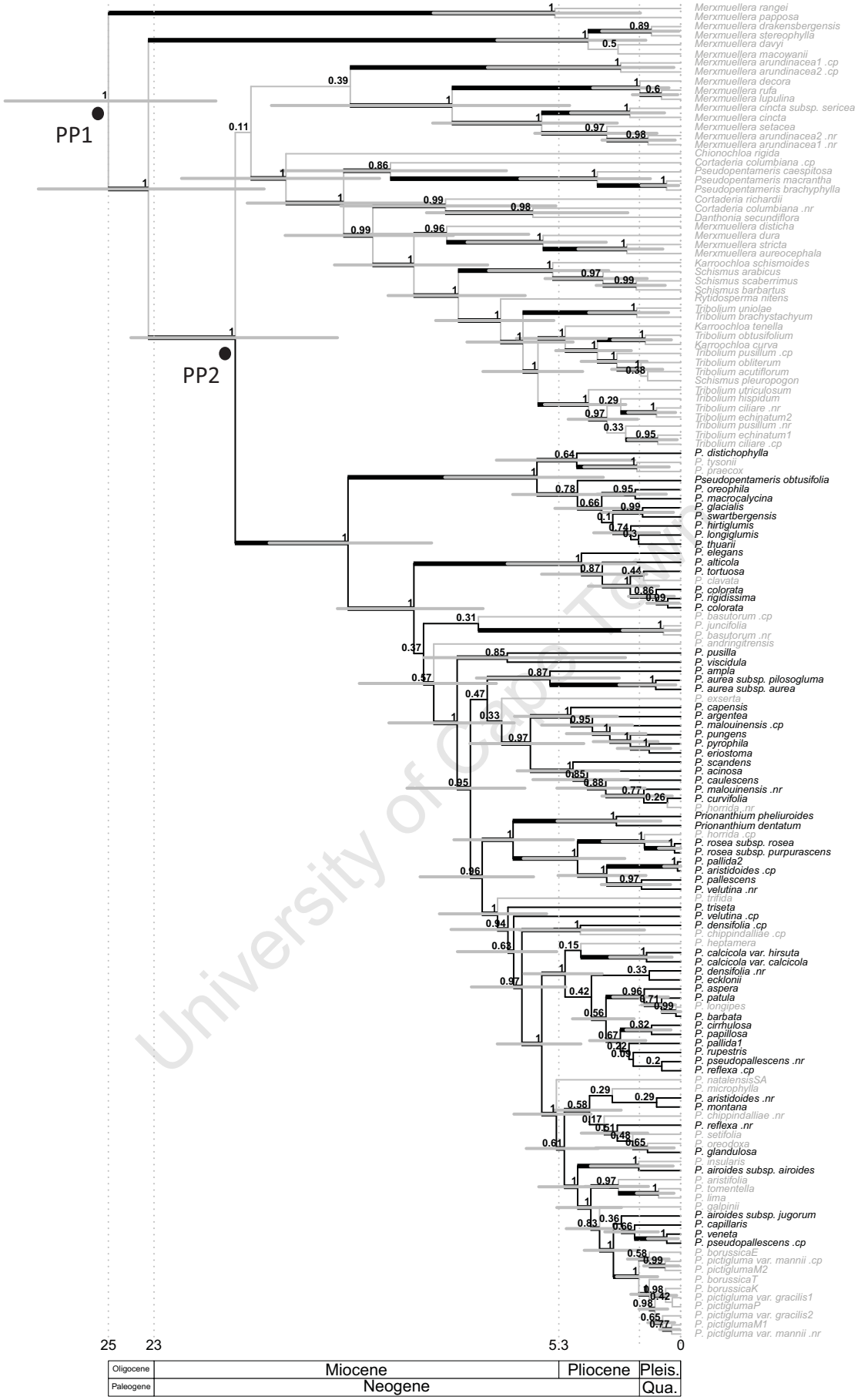

Figure 3.8 Dated Bayesian Maximum clade credibility tree for Danthonioideae (*Pentameris*). Numbers on nodes represent posterior probability support values. Calibration node are indicated by `PP1' and `PP2' as in the higher-level phylogeny (Poales) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region. Nuclear and plastic accessions are denoted with '.nr' and '.cp', respectively.

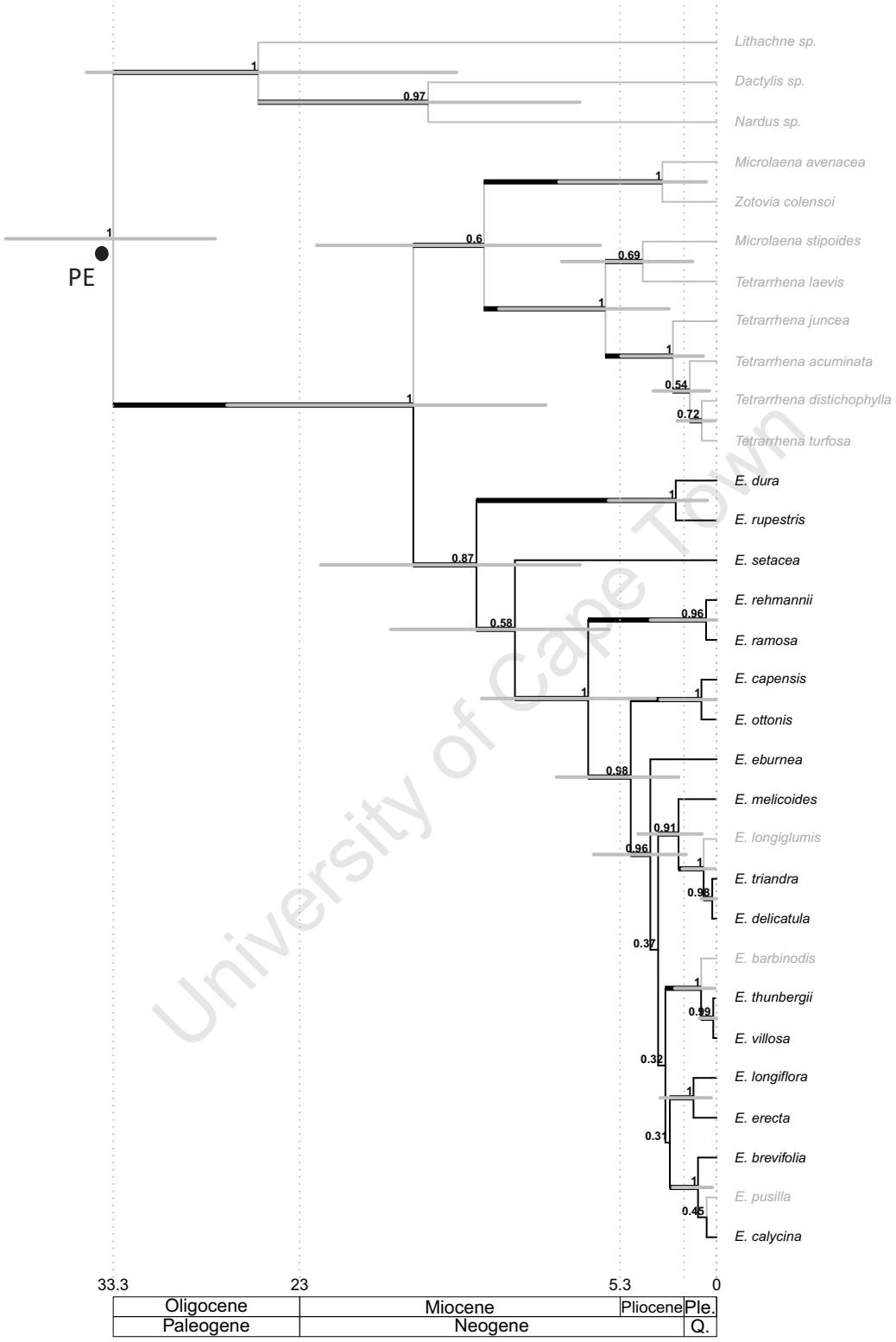

Figure 3.9 Dated Bayesian Maximum clade credibility tree for *Ehrharta*. Numbers on nodes represent posterior probability support values. The calibration node is indicated by `PE' as in the higher-level phylogeny (Poales) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

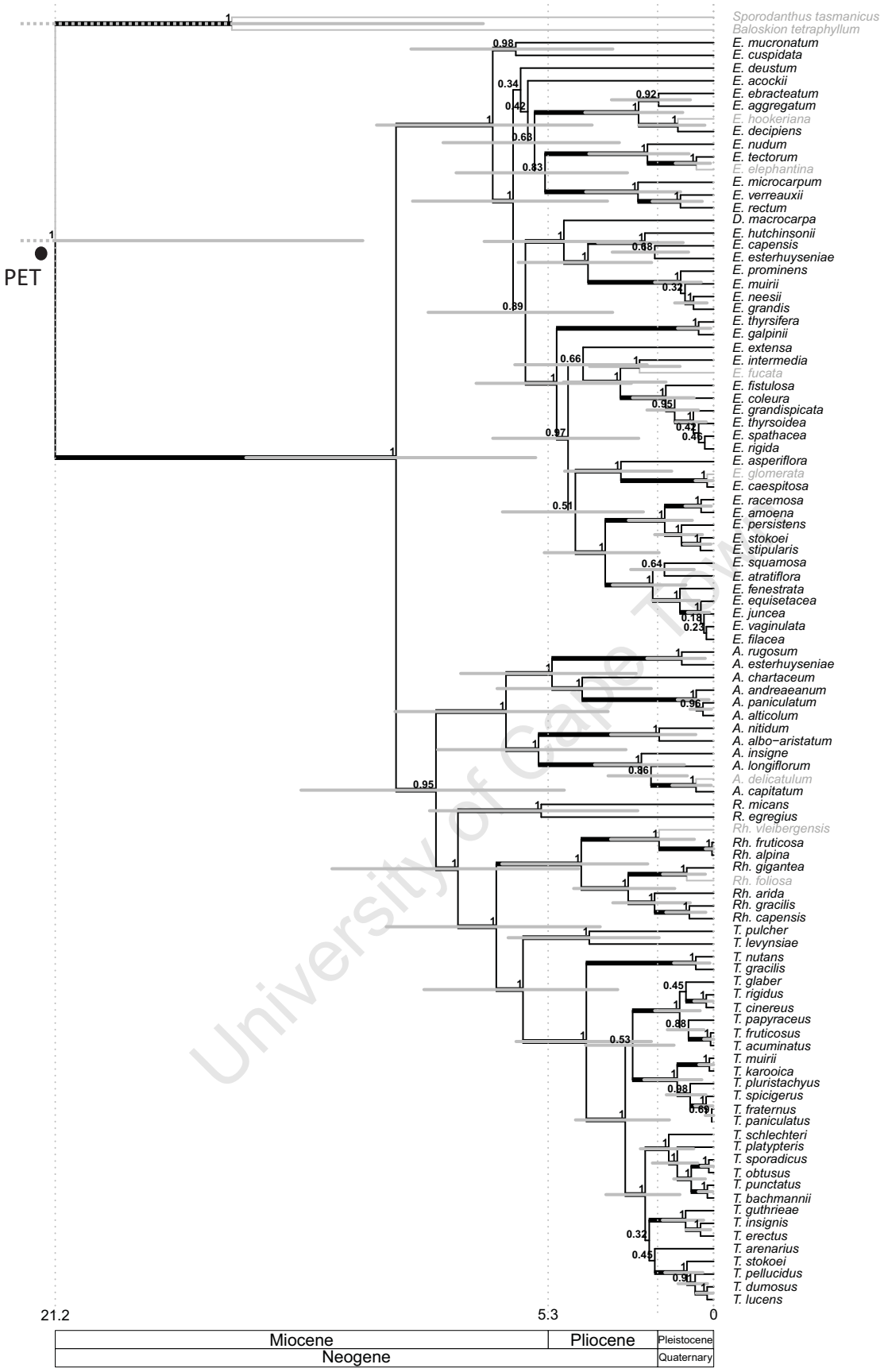

Figure 3.10 Dated Bayesian Maximum clade credibility tree for the *Elegia/Thamnochortus* clade. Numbers on nodes represent posterior probability support values. The calibration node is indicated by `PET' as in the higher-level phylogeny (Poales) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

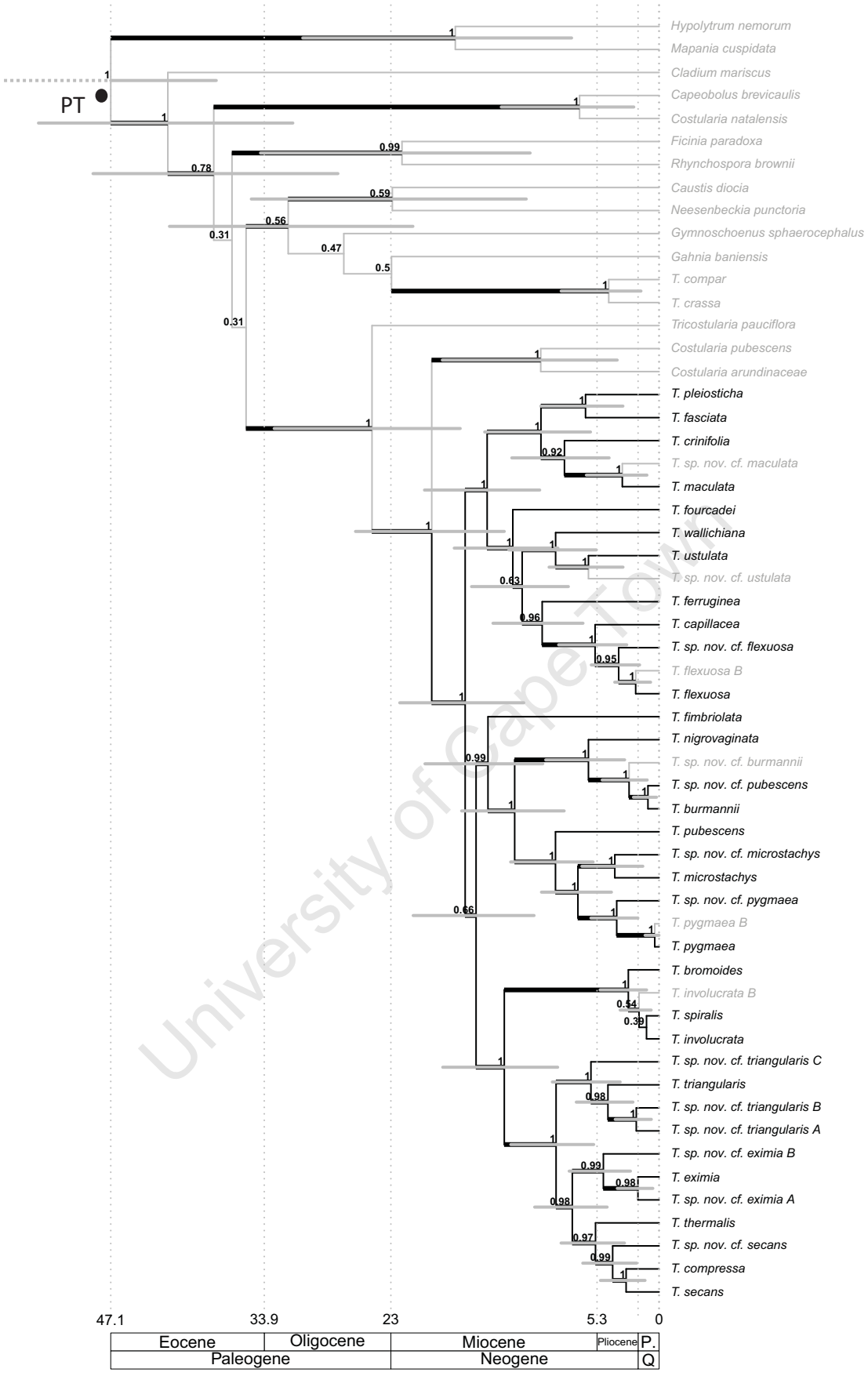

Figure 3.11 Dated Bayesian Maximum clade credibility tree for *Tetraria*. Numbers on nodes represent posterior probability support values. The calibration node is indicated by `PT' as in the higher-level phylogeny (Poales) from which the calibration was obtained (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

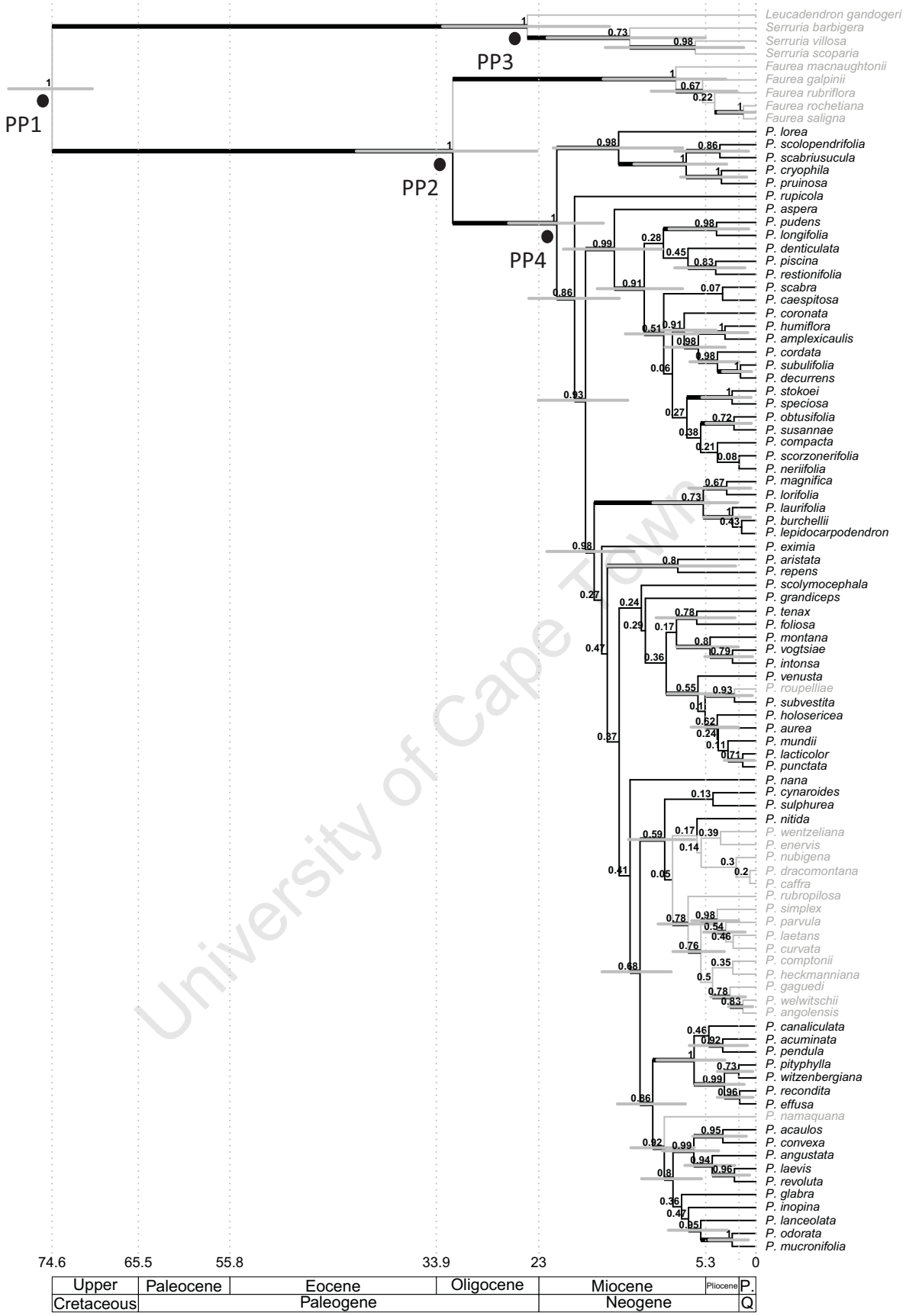

Figure 3.12 Dated Bayesian Maximum clade credibility tree for *Protea*. Numbers on nodes represent posterior probability support values. Calibration nodes are indicated by `PP1-4', and were obtained from Sauquet *et al.* (2009) (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

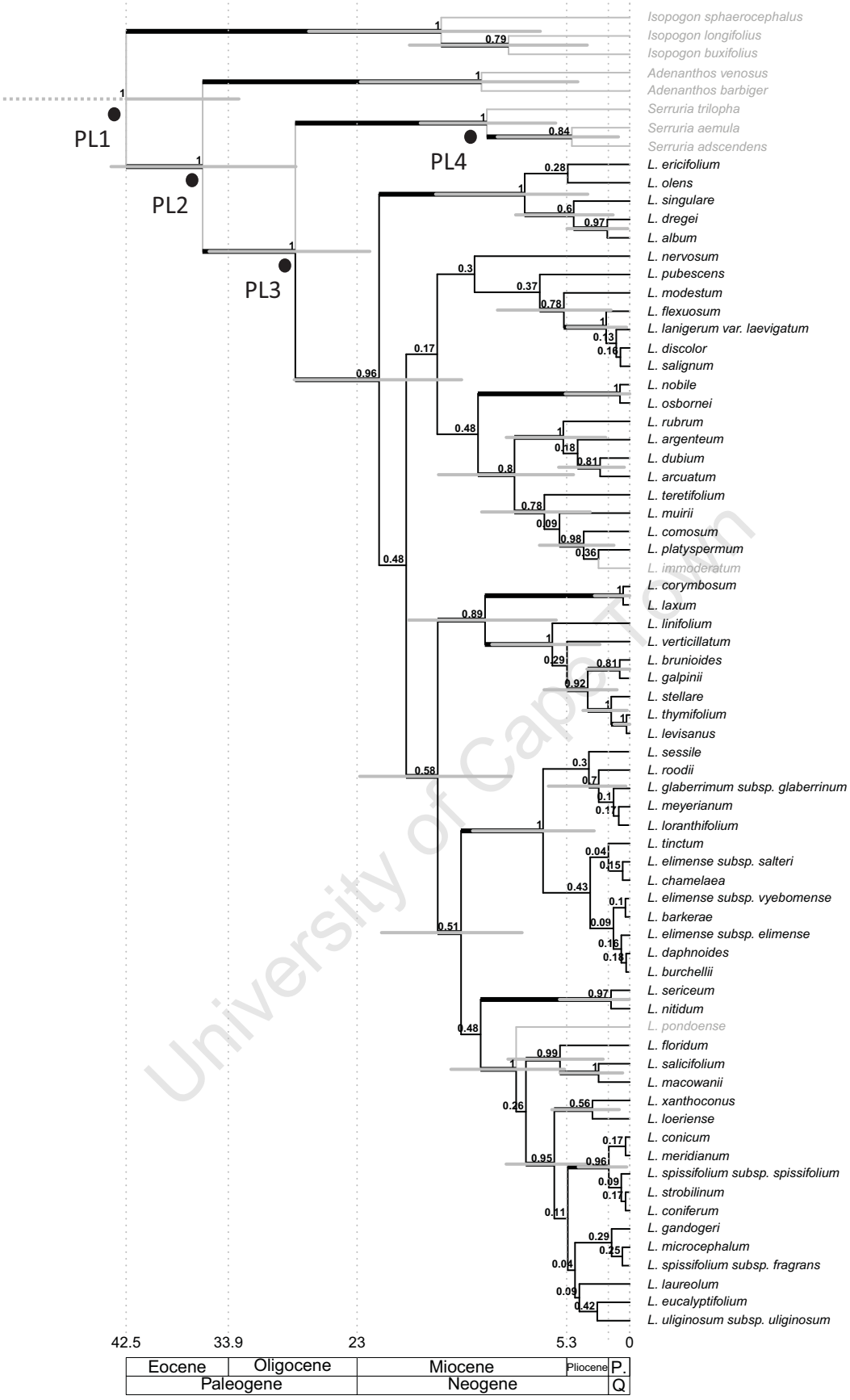

Figure 3.13 Dated Bayesian Maximum clade credibility tree for *Leucadendron*. Numbers on nodes represent posterior probability support values. Calibration nodes are indicated by `PL1-4', and were obtained from Sauquet *et al.* (2009) (see Table 2.3 for detailed information on calibration ages). Grey-coloured taxa represent taxa for which no georeferenced data was available or which do not occur in the Cape Floristic Region.

Protea). For the remaining 11 comparisons, age discrepancies (the difference between previously published and new age estimates, expressed as a percentage of the former) ranged from -68.7 to 24.5%. In contrast to the clock model applied in this study (as employed in BEAST), most previous studies made use of dating methods which assume rate autocorrelation (PL, NPRS and Multidivtime), these giving dates that were consistently older (Figure 3.14). Where previously published age estimates were obtained using BEAST, however, age estimates were both older and younger. The mean percentage discrepancy in age estimates differed significantly between instances where the previous dates were obtained using methods that assume rate autocorrelation (PL, NPRS and Multidivtime) versus those that did not (BEAST) ($t = 2.51$, $df = 12$, $p < 0.05$; Figure 3.14).

Table 3.2 Comparison of molecular age estimates obtained in this study against age estimates obtained from previous dating analyses. Each discrepancy is expressed as the difference between previously published and new age estimates, expressed as a percentage of the former. N is the number of nodes used for each comparison. For negative differences, the nodes in the previous study were older, and vice versa for positive differences (see also Figure 3.14 for more details). Symbols that are given next to 'Method' refer to different calibration types (* = fossil, † = secondary calibration, ^{ext} = external rates, ^ = biogeographic constraint).

Group	Method	N	Discrepancy (%)	Study
			Mean [Range]	
Orchidaceae	PL*	7	-22.8 [-6.8 - -31.7]	Ramirez *et al.* 2007
Orchidaceae	NPRS*	7	-27.2 [-5.7 - -49.4]	Ramirez *et al.* 2007
Orchidaceae	BEAST*	7	-13.6 [-2.6 - -21.8]	Gustafsson *et al.* 2010
Poales	NPRS*	5	-34.4 [-10.6 - -63.5]	Janssen & Bremer 2004
Poales (Poaceae)	Multidivtime*	2	-25.6 [-21.0 - -30.2]	Vicentini *et al.* 2008
Poales (Poaceae)	Multidivtime*	5	-18.1 [5.1 - -37.4]	Christin *et al.* 2008
Poales (Poaceae)	BEAST*	10	-30.2 [35.3 - -55.0]	Bouchenak-Khelladi *et al.* 2010
Asteraceae	NPRS*	3	-22.9 [-5.21 - -45.1]	Kim *et al.* 2005
Arctotidinae	BEAST^{ext}	5	24.5 [25.8 - -29.5]	McKenzie & Barker 2008
Stoebe	BEAST†^	4	-37.2 [-27.8 - -46.2]	Bergh & Linder 2009
Ehrharta	NPRS†	2	-59.7 [-56.5 - -62.9]	Verboom *et al.* 2003
Elegia/Thamnochortus	Multidivtime†	8	-68.7 [-66.0 - -71.0]	Linder *et al.* 2005
Tetraria	BEAST*	4	10.5 [7.6 - 13.9]	Slingsby 2011
Protea	BEAST†	6	13.6 [32.9 - -50.5]	Valente *et al.* 2009

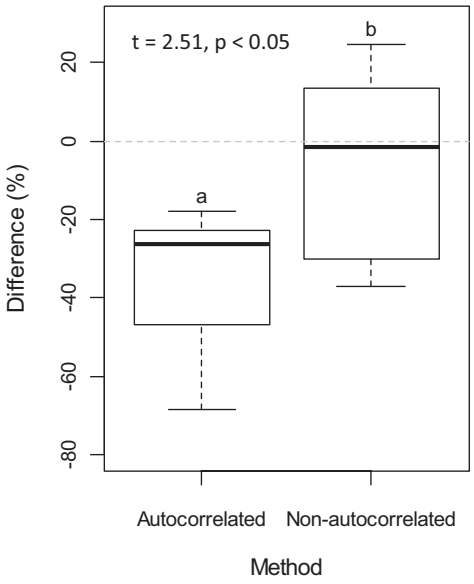

Figure 3.14 The percentage discrepancy between previously published age estimates obtained using either autocorrelated (PL, NPRS, Multidivtime) or non-autocorrelated (BEAST) methods, and age estimates obtained in this study (using a non-autocorrelated method, BEAST). Each discrepancy is expressed as the difference between previously published and new age estimates, expressed as a percentage of the former. The mean age discrepancy between autocorrelated and non-autocorrelated methods was significantly greater than the mean difference in age estimates between autocorrelated methods ($t = 2.51$, $p < 0.05$). Age esimtates obtained using methods that assume rate autocorrelation were consistently older than age estimates obtained using methods that do not assume rate autocorrelation.

3.2 Patterns of habitat endemism

3.2.1 Seasonality

Of the 454 taxa, 81% were endemic to either aseasonal or seasonal habitats. Of these, approximately 86% occurred in aseasonal environments and 14% occurred in strictly seasonal habitats. The highest percentage of seasonal-endemics was found in *Ehrharta* (~23%). Two of the groups, *Tetraria* and *Satyrium*, occurred only in aseasonal and aseasonal/seasonal habitats (i.e. no seasonal-endemics).

3.2.2 Mean annual precipitation

Approximately 55% of all taxa were classifiable to one of the four pre-defined MAP categories. Of these, 56% were restricted to 300 – 599 mm/year, 29% to 600 – 899 mm/year, 8% to > 900 mm/year, and 7% to < 300 mm/year. The remaining 45 % (non-endemics) occurred across two or more MAP categories. The highest numbers of MAP endemics were found in *Leucadendron* (~ 75%) and *Stoebe* (~ 67%). For both of these, the majority of endemics were restricted to 300 – 599 mm/year.

3.2.3 Substrate type

Approximately 67% of the 454 taxa were endemic to a specific substrate type. The percentage of substrate endemics in each group, in turn, ranged from 23% in Arctotidinae to as high as 85% in *Tetraria*. Of all substrate endemics, approximately 80% were endemic to quartzite (this ranged between 19 and 85% within each group), 7% to shale substrates, 7% to calcareous deposits, 4% to lowland sands, and less than 2% to alluvial deposits. No taxa were found to be endemic to granite substrates. The remaining taxa were mostly polymorphic for two (or more) substrates, generally involving a combination of quartzite and either alluvial/calcareous deposits or shale substrates.

3.2.4 Vegetation type

The majority of extant taxa were found to be endemic to fynbos vegetation (~72%) with only 2% being endemic to renosterveld, strandveld or succulent karoo vegetation. The remaining 26% of taxa were scored as polymorphic, occurring in two or more vegetation types. A maximum of two renosterveld, strandveld or succulent karoo endemics were found in any one group. The percentage of fynbos endemics within groups ranged from 33% to 94% (*Satyrium* and *Tetraria*, respectively). Of the 120 taxa not coded as endemic to a particular vegetation type, most were polymorphic for fynbos and renosterveld vegetation (~57%), with 10% polymorphic for fynbos and strandveld, and 5% fynbos and succulent karoo. The remaining non-endemics (~28%) were polymorphic for three or four vegetation types.

3.2.5 Assessment of GIS-based habitat classification

Amongst the six groups selected for comparison of GIS- and expert-based scoring of substrates, the percentage of species for which the two scoring methods yielded identical results varied from 35.3 to 88.9%, with a mean of 57.8% (Table 3.3). With the exception of *Pentameris* and *Ehrharta*, the percentage of instances in which the two methods gave different (non-nested) results was consistently low (< 10%). The majority of non-identical expert-based scorings were either broader or narrower than the GIS-based scorings (on average 13.5% in either case). On average, only 5.7% of expert-based scorings were entirely different to GIS-based scorings.

Table 3.3 Comparisons between expert- and GIS-based substrate scoring for six groups with differences calculated as the percentage of taxa for which scoring was identical. Given are the number of taxa for which comparisons were made (N), subdivided into four main categories. 'Identical' describes cases for which substrate scoring was the same. 'Broader' describes cases in which GIS-scoring was a subset of the expert-based scoring (and vice versa for 'Narrower'). 'Different' describes two situations: 'Nested' where expert- and GIS-based scoring were the same for one or more substrate types, but differed in the remaining states (i.e. quartzite+alluvial versus quartzite+shale+lowsands), and 'Non-nested' where substrate scoring was entirely different.

		Expert scoring versus GIS scoring				
Group	N	Identical	Broader	Narrower	Different	
					Nested	Non-nested
Stoebe	45	40 (88.9%)	1 (2.2%)	1 (2.2%)	1 (2.2%)	2 (4.4%)
Pentameris	63	37 (58.7%)	8 (12.7%)	5 (7.9%)	2 (3.2%)	11 (17.5%)
Ehrharta	17	6 (35.3%)	1 (5.9%)	3 (17.6%)	6 (35.3%)	1 (5.9%)
Elegia/Thamnochortus	88	55 (62.5%)	8 (9.1%)	16 (18.2%)	7 (8%)	2 (2.3%)
Protea	69	32 (46.4%)	18 (26.1%)	14 (20.3%)	2 (2.9%)	3 (4.3%)
Leucadendron	60	33 (55%)	15 (25%)	9 (15%)	3 (5%)	0 (0%)
Average		57.8%	13.5%	13.5%	9.4%	5.7%
					7.6 %	

3.3 Patterns of habitat shifts

The majority of divergence events occurred in aseasonal, semi-arid to mesic conditions and on quartzitic soils (Figure 3.15). Divergences within the other habitats (seasonal, arid, non-quartzite) occurred infrequently and predominantly in the more recent past.

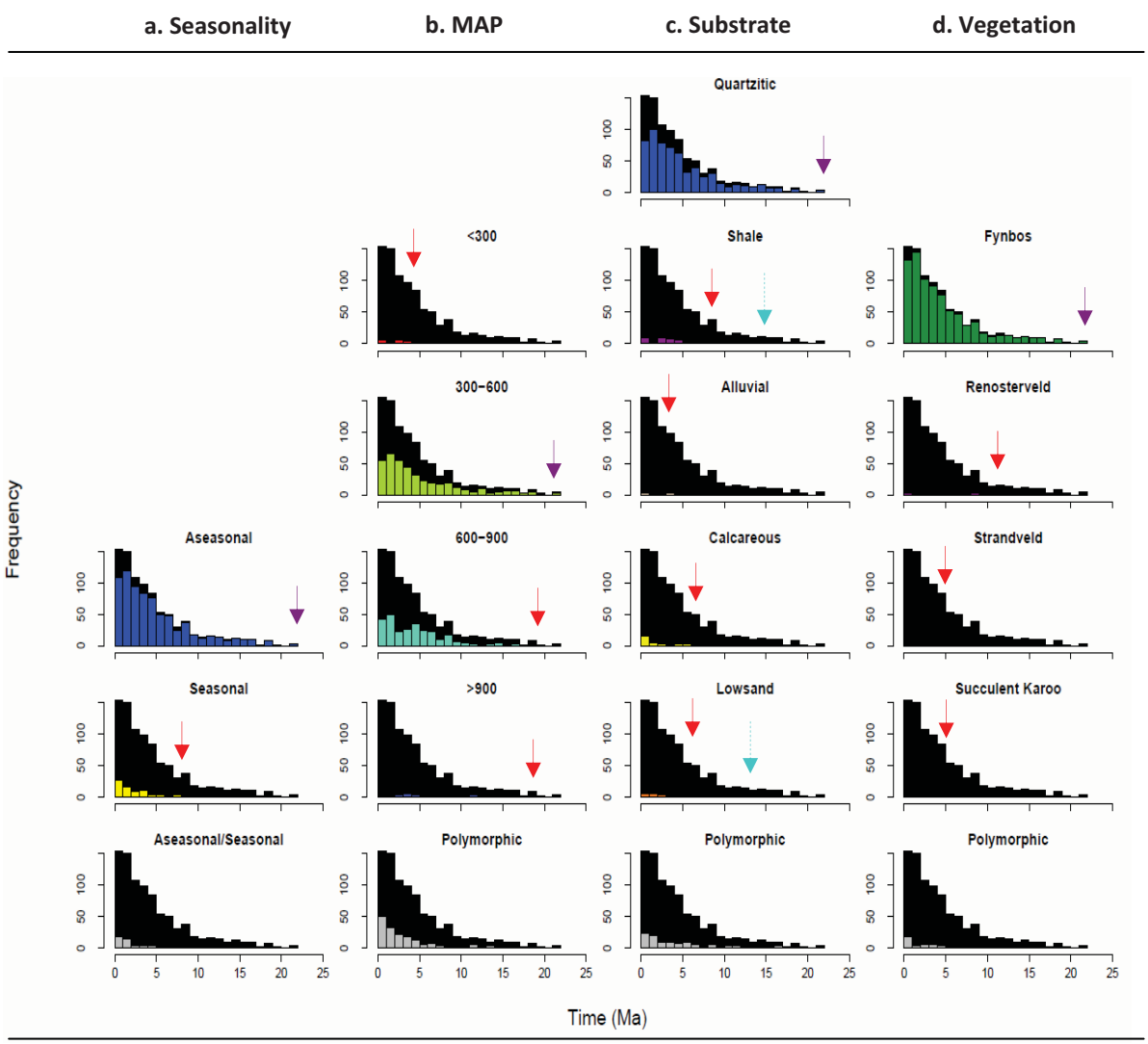

Figure 3.15 Frequency histograms of combined divergence events across all 11 groups from the earliest divergence (~21 Ma) to present. Black-coloured histograms depict all divergence events, coloured histograms depict the number of divergence events that occurred within each of the pre-specified environmental classes, a. seasonality, b. MAP, c. substrate, and d. vegetation type. Earliest shifts into each class are depicted by red arrows, purple coloured-arrows depicting the class that was most commonly reconstructed as the ancestral condition across the 11 groups. Light-blue arrows depict cases of very early shifts to shale- and lowsands-endemism that are questionable based on conflict between GIS- and expert-based substrate scoring.

The ancestral nodes for 10 of the 11 groups (excluding Arctotidinae) were reconstructed as endemic to an aseasonal, semi-arid to mesic environment underlain by oligotrophic quartzitic soils (Figures 3.16 to 3.19). The root node of Arctotidinae was assigned to a similar environment, except that it was reconstructed as polymorphic with respect to substrate type. Also, in contrast to the other 10 groups which were ancestrally reconstructed as endemic to fynbos vegetation, the root node of Arctotidinae was reconstructed as endemic to renosterveld vegetation (Figure 3.19).

The overall pattern of seasonality shifts was unidirectional, with shifts occurring from an ancestrally aseasonal to seasonal or seasonally-polymorphic environment (Figure 3.16). A similar trend was observed in substrate and vegetation occupancy, with most transitions involving shifts from quartzitic substrate endemism to mostly polymorphic substrate occupancy (Figure 3.18), and from fynbos endemism to polymorphic vegetation-type occupancy (Figure 3.19). Reversals to the ancestral, wide-spread habitat states (e.g. aseasonal, quartzitic, fynbos) were rare. Transitions across MAP gradients varied quite substantially between groups and consistent patterns were difficult to discern across all eleven groups (Figure 3.17). The most common pattern, however, involved shifts from ancestrally mesic to either more arid or wetter environments.

Comparisons of habitat shifts across the two conflicting nuclear and plastid data sets for *Pentameris* indicated no difference in age estimates for endemism shifts based on ancestral state reconstructions (Table 3.4). Hence, only the reconstructions based on the tree containing the plastid accessions of conflict taxa were used for subsequent statistical analyses.

3.3.1 Seasonality

The ancestral node was reconstructed as endemic to an aseasonal habitat for all 11 groups (Figure 3.15). Shifts across the seasonality classes generally occurred from an ancestrally aseasonal-endemic to either a seasonal-endemic or to a seasonality-generalist state. With the exception of *Tetraria* and *Satyrium*, which did not contain any seasonal-endemics, age estimates for the earliest shift to seasonal-endemism in each group ranged from 0.30 Ma (*Disperis*) to 13.35 Ma (*Protea*), with a mean of 5.41 Ma (Table 3.4). Across all groups, the age estimates for

the earliest divergence of seasonal-endemics were consistently younger than age estimates for the earliest divergences of aseasonal-endemics (paired t = 6.0617, p < 0.001; see Figure 3.20). The mean earliest shift to seasonal-endemism across all groups did not differ significantly from the proposed timing of 5 Ma that marks the intensification of seasonal conditions in response to tectonic uplift (t_{5Ma} = 0.3132, p = 0.7621; Linder 2003), but did differ from the proposed timing of 10 to 14 Ma marking the onset of large-scale aridification linked to the establishment of the Benguela upwelling system (t_{10Ma} = -3.5277, p < 0.01; t_{14Ma} = -6.6004, p < 0.001; Siesser 1978, 1980; Diester-Haass *et al.* 2002).

In about 12 cases, the occupation of seasonal environments (shift to either seasonal-endemism or aseasonal/seasonal habitat occupation) by a descendent lineage led to clade diversification (here defined as number of taxa > 3) in the new environment. Nevertheless in seven of these, reversals to aseasonal-endemism were also observed.

Table 3.4 Summary of the estimated ages (Ma) of the earliest appearance of habitat endemism within each group with respect to habitat (a) seasonality and (b) substrate type. Asterisks (*) represent divergences of single species scored as shale-endemic by GIS-scoring, but scored as quartzite-endemics by expert-scoring, with ages in brackets representing the earliest divergences if the former (GIS-Scored taxa) are excluded. In both cases, the number of records was very small. In the case of *Stoebe*, the earliest shale divergence (marked with †) was included here based on expert-opinion scoring that identified it as a shale-endemic (GIS-based scoring identified this as a shale-lowsands species). For *Pentameris*, age estimates for both the plastid (CP) and nuclear (NR) data sets are provided.

Group	a. Seasonality		b. Substrate				
	Aseasonal	Seasonal	Quartzite	Shale	Calcrete	Alluvial	Lowsands
Arctotidinae	11.91	1.71	5.66			3.21	
Stoebe	7.10	4.68	7.10	1.91†	1.84		3.63
Disperis	18.40	0.27	18.40	5.51			
Pterygodium	18.79	6.48	18.79	6.48			3.12
Satyrium	10.31		10.31		6.36		
Pentameris CP	14.55	7.56	14.55	8.01* [4.34]	5.06		2.81
Pentameris NR	14.55	7.56	14.55	8.01* [4.34]	5.06		2.81
Ehrharta	12.30	2.10	12.26	4.75			
Elegia/Thamnochortus	11.20	5.98	11.23	4.21	2.44	0.60	5.98
Tetraria	16.65		16.65				
Protea	21.12	13.35	21.12	15.02* [4.26]	5.86	3.51	12.16
Leucadendron	21.17	6.54	21.17	5.56	6.54	1.99	1.56
Average	14.84	5.41	14.32	6.45 [4.64]	4.74	2.33	4.58

3.3.2 Mean annual precipitation

The ancestral node for seven of the 11 groups was reconstructed as endemic to a relatively mesic environment receiving about 600 mm/year or more (*Ehrharta*, *Tetraria*, *Elegia/Thamnochortus*, *Disperis*, *Pterygodium*, *Satyrium* and *Pentameris;* see Figure 3.16). Arctotidinae, *Stoebe* and *Leucadendron* were reconstructed as originating in drier conditions, at 300 to 600 mm/year. Despite the root node of *Ehrharta* being reconstructed as polymorphic with respect to MAP, closer inspection of the polymorphic state reveals that the root node for this group occurred in relatively wet conditions (600 to > 900 mm/year). In *Protea*, the ancestral lineage was polymorphic for MAP, being reconstructed to range across both dry and wet environments, i.e. 300 to 900 mm/year.

For MAP, consistent directional trends in habitat shifts were difficult to discern, most likely because shifts between the three categories describing the more mesic end of the MAP spectrum were relatively common (i.e. 300 – 600, 600 – 900, > 900 mm/year). The mean ages of the earliest shifts to endemism across these three mesic categories were not significantly different, but the mean age for earliest shift to arid-endemism (< 300 mm/year) was significantly younger than mean ages for the earliest shifts to 300 – 600 and 601 – 900 mm/ year categories (Kruskal-Wallis $\chi^2 = 12.69$, df = 3, $p < 0.01$; Figure 3.20).

Nevertheless, two consistent patterns were found. Firstly, across the mesic-origin clades (> 600 mm/year), MAP shifts generally involved a shift from mesic- to arid-endemism, though a few selected shifts to more mesic conditions (> 900 mm/year) also occurred. Within these groups (*Ehrharta*, *Disperis*, *Tetraria*, *Elegia/Thamnochortus*, *Pentameris* and *Pterygodium*), age estimates for the earliest shifts to semi-arid endemism ranged from 3.66 Ma (*Ehrharta*) to 16.31 Ma ago (*Pterygodium*), with a mean of 9.07 Ma. Secondly, shifts to arid-endemism (< 300 mm/year) were only observed in seven of the 11 groups, within which they occurred infrequently. Age estimates for the earliest shifts to arid-endemism within each of these groups ranged from 0.20 Ma (*Disperis*) to 4.68 Ma (*Stoebe*), with a mean of 2.35 Ma. In contrast to the number of reversals that were observed between the more mesic MAP-categories, there were no reversals to less arid environments once a lineage had shifted to arid-endemism.

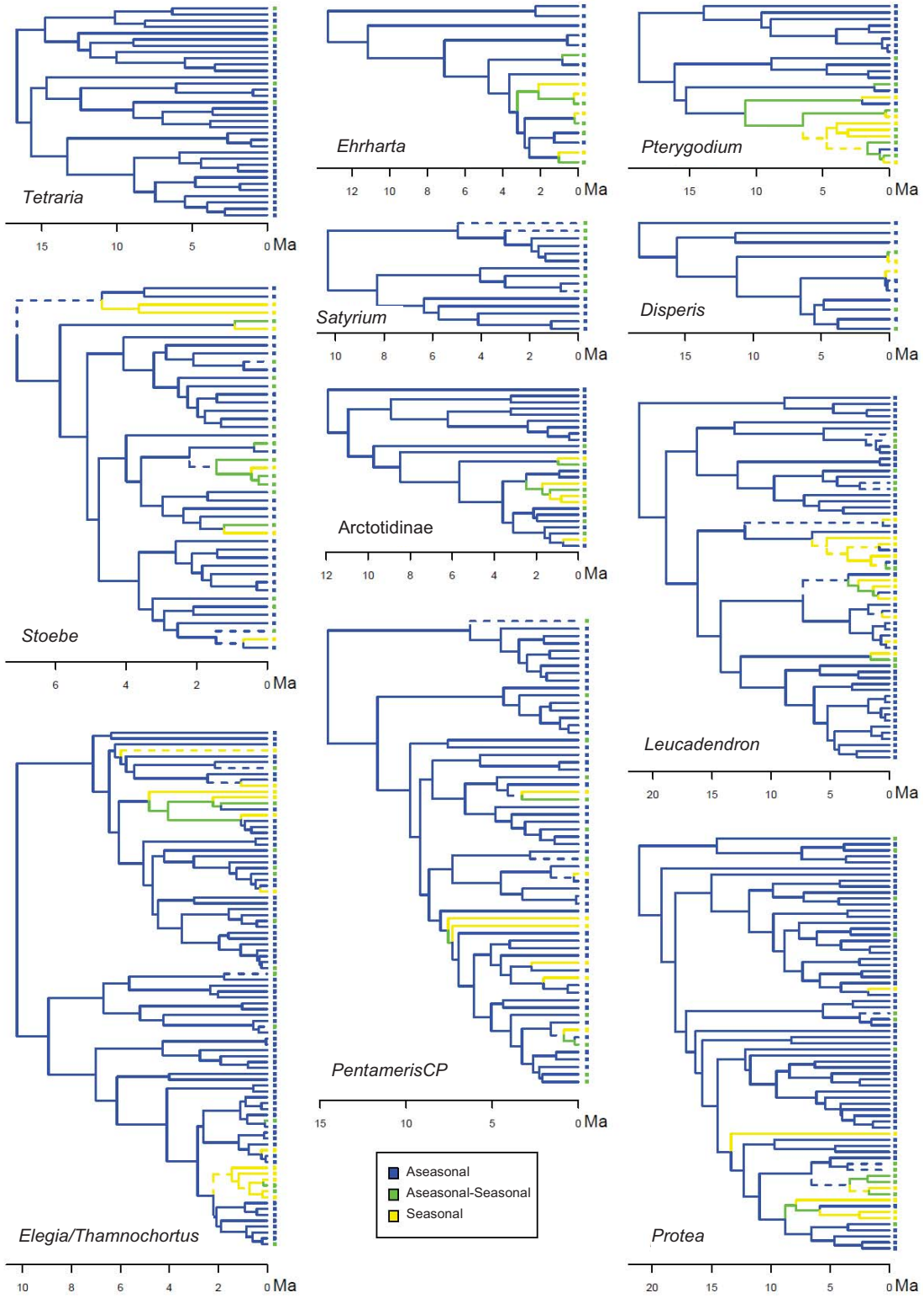

Figure 3.16 Optimization of precipitation seasonality across each of the 11 groups. Filled boxes at the tips of the phylogenies depict the state of the extant taxa. Solid lines depict lineages for which the reconstructed state was estimated as greater than 60%. Dashed lines depict lineages for which the probability of the estimated state was between 50 - 60%. Polymorphic lineages, here depicted as `Aseasonal-Seasonal' were those for which the probability of any state was less than 50%.

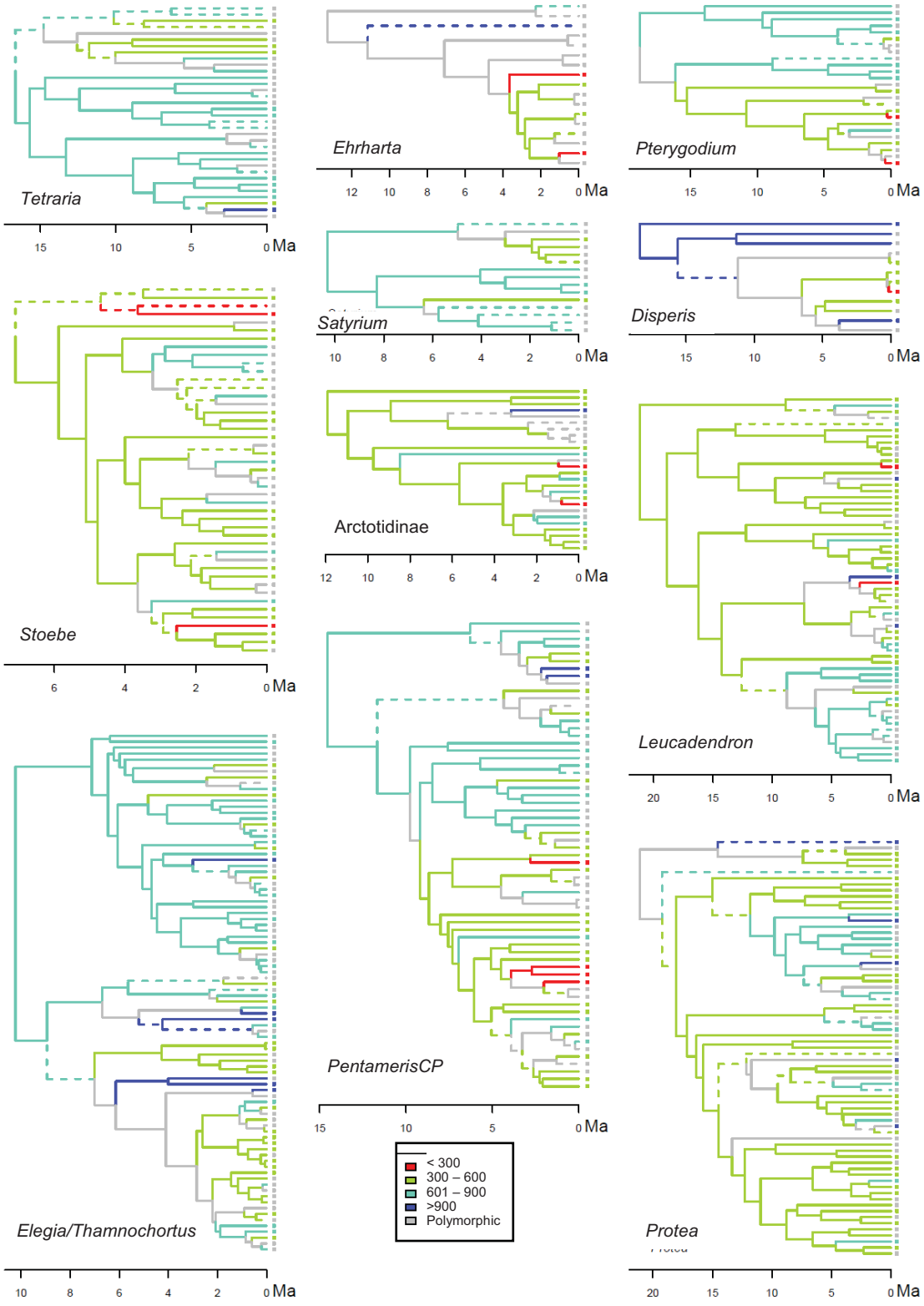

Figure 3.17 Optimization of mean annual precipitation across each of the 11 groups. Filled boxes at the tips of the phylogenies depict the state of the extant taxa. Solid lines depict lineages for which the reconstructed state was estimated as greater than 60%. Dashed lines depict lineages for which the probability of the estimated state was between 50 - 60%. Polymorphic lineages were those for which the probability of any state was less than 50%.

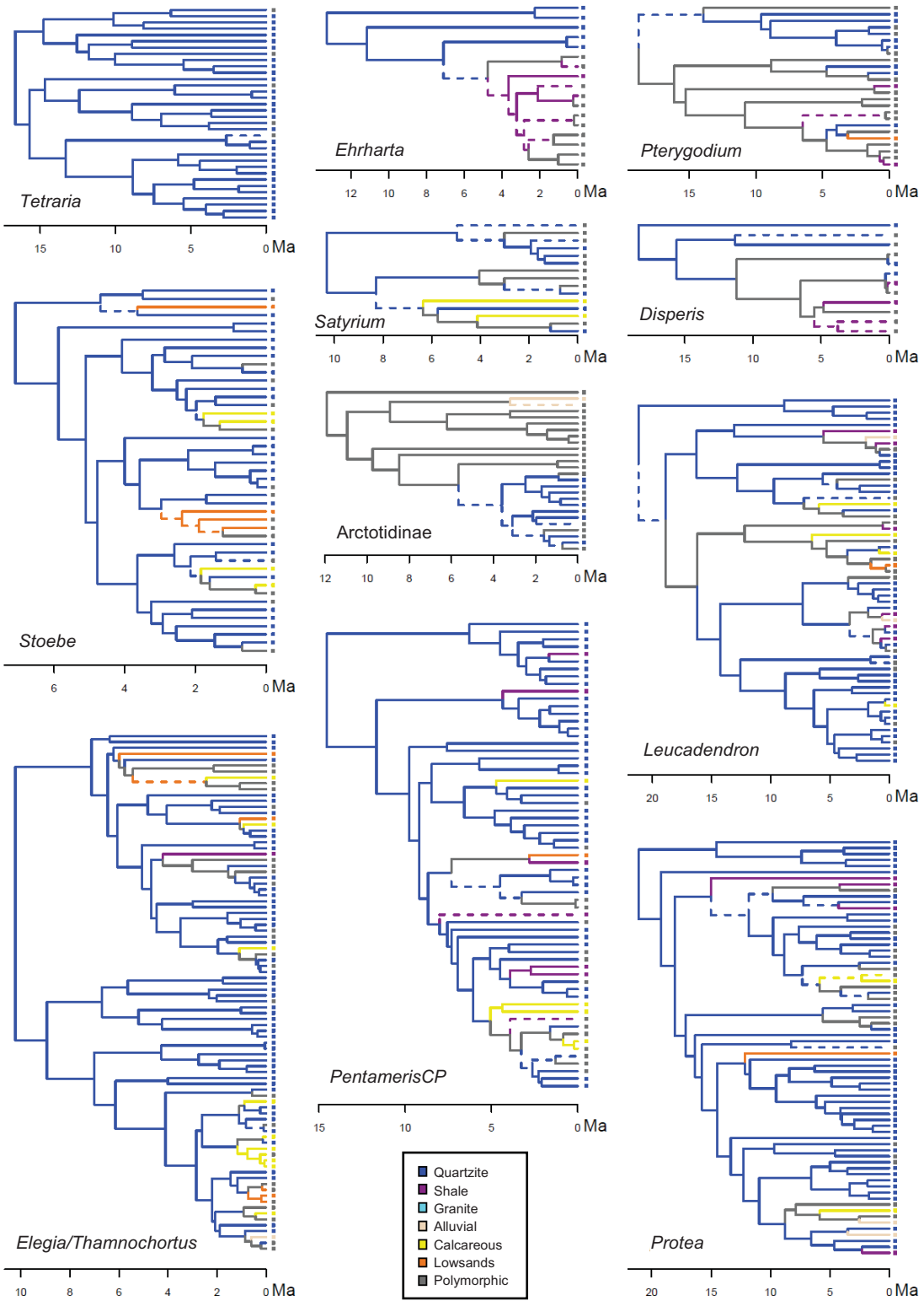

Figure 3.18 Optimization of substrate type across each of the 11 groups. Filled boxes at the tips of the phylogenies depict the state of the extant taxa. Solid lines depict lineages for which the reconstructed state was estimated as greater than 60%. Dashed lines depict lineages for which the probability of the estimated state was between 50 - 60%. Polymorphic lineages were those for which the probability of any state was less than 50%.

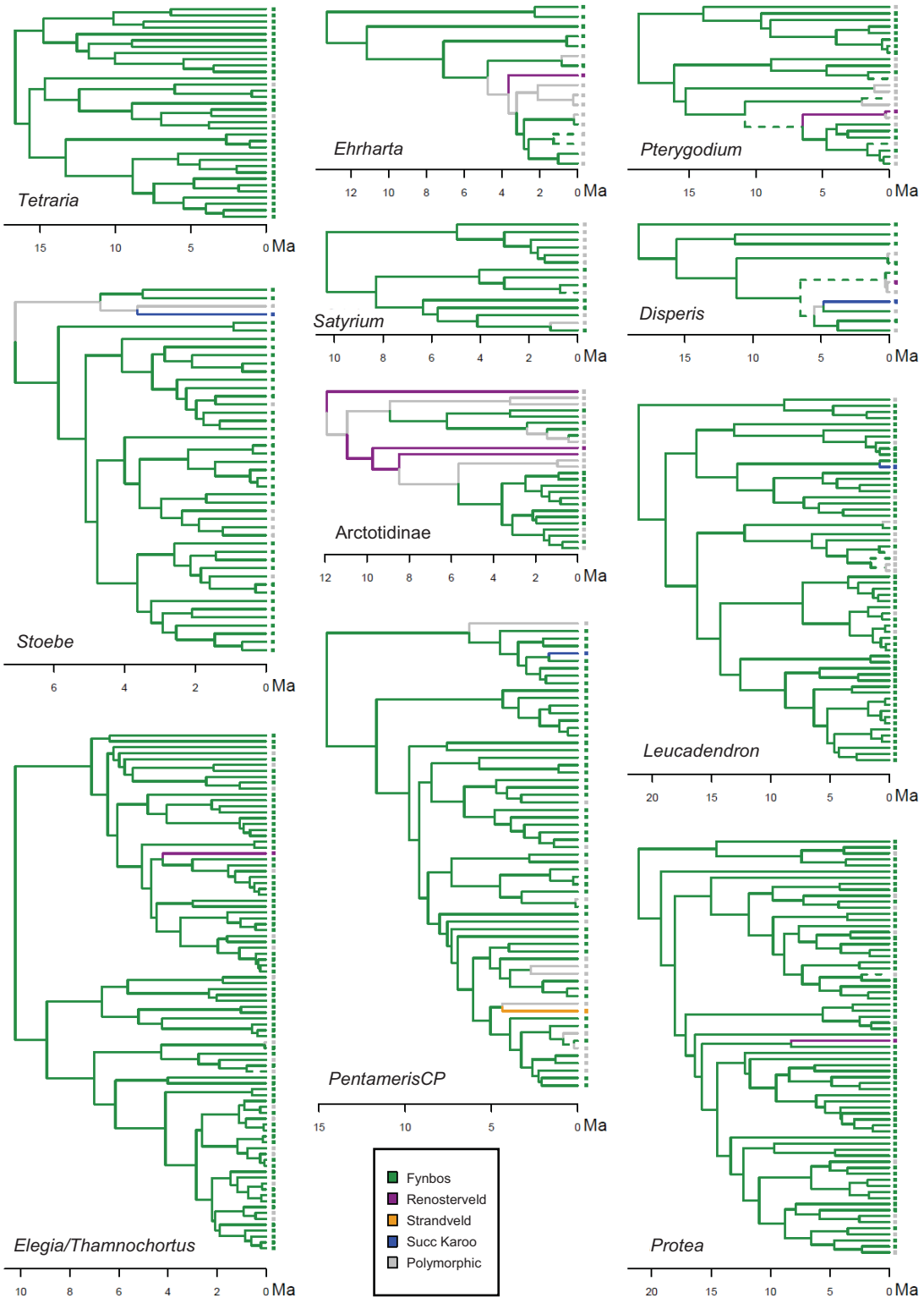

Figure 3.19 Optimization of vegetation type across each of the 11 groups. Filled boxes at the tips of the phylogenies depict the state of the extant taxa. Solid lines depict lineages for which the reconstructed state was estimated as greater than 60%. Dashed lines depict lineages for which the probability of the estimated state was between 50 - 60%. Polymorphic lineages were those for which the probability of any state was less than 50%.

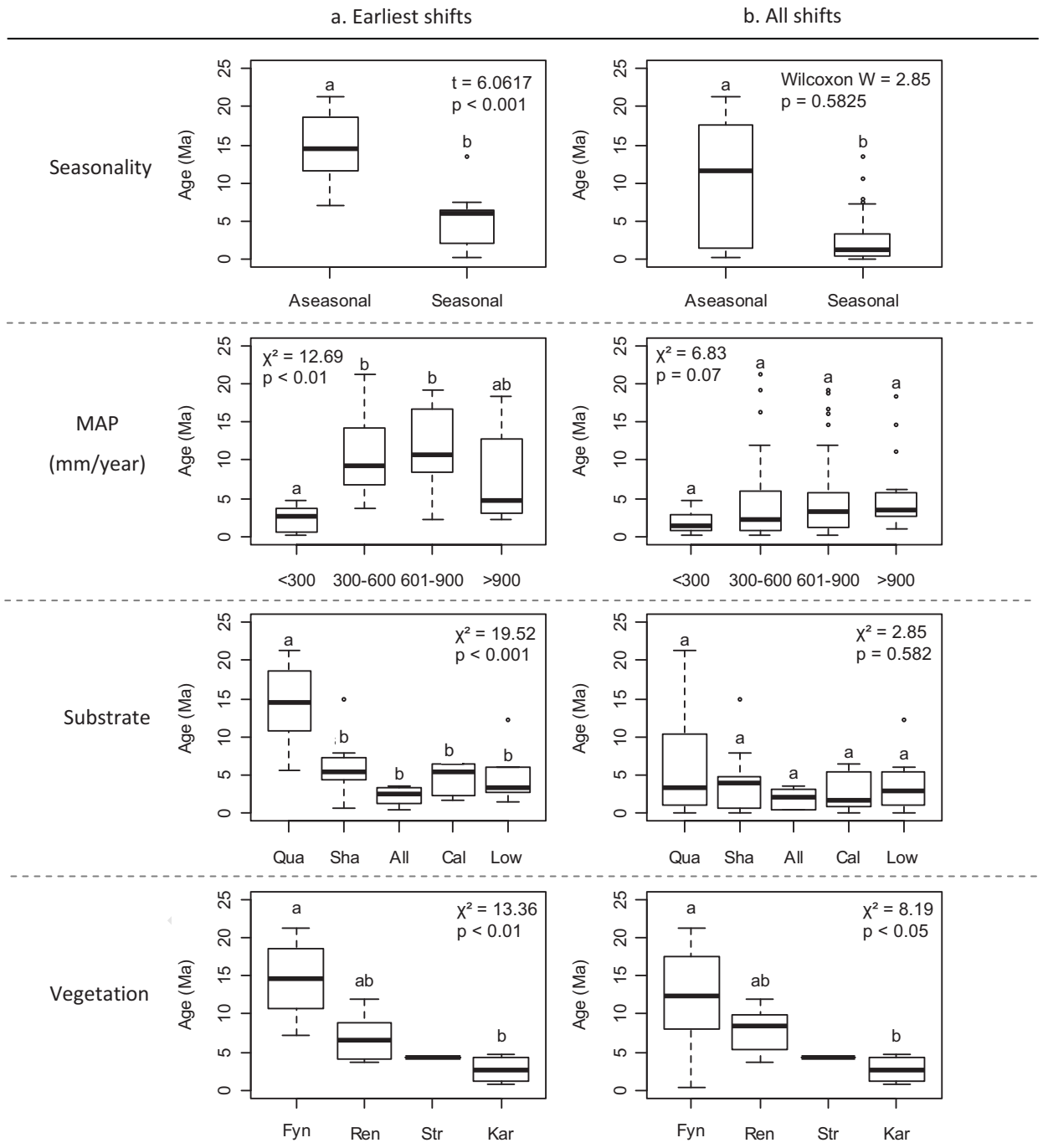

Figure 3.20 The mean ages of habitat endemism shifts across all eleven groups calculated for (a) the earliest shifts, and (b) for all shifts. Letters above boxes denote significant differences at $p < 0.05$. The different habitat categories are given along the x-asis. For substrate, `Qua' = quartzite, `Sha' = shale, `All' = alluvial, `Cal' = calcareous soils, and `Low' = lowsands. For vegetation, `Fyn' = fynbos, `Ren' = renosterveld, `Str' = strandveld, and `Kar' = succulent karoo. W and χ^2 denote test statistics obtained from Wilcoxon rank sum test and Kruskal-Wallis rank rum test, respectively.

Patterns of rainfall transitions across the remaining four groups (*Stoebe*, Arctotidinae, *Leucadendron* and *Protea*) which were reconstructed as ancestrally having occupied less mesic habitats (300 – 600 mm/year), were relatively inconsistent with several shifts occurring both to higher and to lower MAP categories. With the exception of Arctotidinae, there is a shift to more mesic conditions (600 – 900 mm/year) within each of these groups that gave rise to a clade consisting largely of mesic-endemics at 3.21, 8.77 and 11.85 Ma (*Stoebe*, *Leucadendron* and *Protea*, respectively).

3.3.3 Substrate type

Quartzite-endemism was reconstructed as the ancestral edaphic state for 10 of the 11 groups in this study (all except Arctotidinae; Figure 3.14). Within these 10 groups, date estimates for the oldest unambiguous transitions from quartzite-endemism to non-quartzite endemism (i.e. a shift to either a non-quartzite monomorphic or to a polymorphic-substrate state) ranged from 3.63 to 18.88 Ma (*Stoebe* and *Leucadendron*), with a mean of 8.79 Ma. Similarly, oldest date estimates for transitions from an ancestral monomorphic quartzite state to a monomorphic non-quartzite state range from 3.63 to 15.02 Ma (*Stoebe* and *Protea*), in each instance involving a switch from quartzite-endemism to shale-, alluvial-, calcrete-, or lowsands-endemism. The one group for which these patterns did not hold in the strictest sense was Arctotidinae, whose ancestral node was reconstructed as being polymorphic with respect to substrate. In this group, the earliest shifts onto quartzitic substrates from a polymorphic ancestor occurred at 5.66 Ma ago.

Earliest unambiguous shifts to shale endemism within each group ranged from 1.91 Ma (*Stoebe*) to 15.02 Ma (*Protea*), with a mean across all groups of 6.45 Ma. Within each group, age estimates for the earliest divergence of shale endemics was significantly younger than age estimates for earliest divergence of quartzite-endemics (paired $t = 6.6882$, $p < 0.001$). Since the two oldest ages for shale-endemics (15.02 Ma and 8.01 Ma) are represented by single species (*Protea aspera* and *Pentameris trifida*, respectively) that were scored as shale-endemics using GIS-based method, but as quartzite-endemics based on expert-scoring, and because each of them was only represented by two records, these two were excluded for subsequent analyses. With the exclusion of these two species the earliest divergence of a shale-endemic decreases to

6.48 Ma (*Pterygodium*). As above, within each group, age estimates for the earliest divergence of shale endemics was significantly younger than age estimates for earliest divergence of quartzite-endemics (paired t = 7.3591, p < 0.0001). The mean age for the earliest shifts to shale-endemism across all clades was significantly different to the proposed time of tectonic uplift at 3 Ma (t_{3Ma} = 3.3654, p < 0.05), but was not significantly different to the earlier date of 5 Ma (t_{5Ma} = -0.7958, p = 0.4523).

Shifts to calcrete-endemism were reflected by a number of taxa within six groups (*Elegia/Thamnochortus*, *Leucadendron*, *Pentameris*, *Protea*, *Satyrium*, and *Stoebe*). Age estimates for the earliest transitions to calcrete endemism in each group ranged from 1.84 Ma (*Stoebe*) to 6.36 and 6.54 Ma (*Satyrium* and *Leucadendron*), with a mean of 4.74 Ma across all groups. Noteworthy is the slightly bimodal distribution of initial shifts to calcrete endemism with a small peak around 5 to 7 Ma ago, followed by a pulse of shifts to calcrete endemism in the more recent past (Figure 3.15). Within each group, age estimates for the earliest divergence of calcrete-endemics was significantly younger than age estimates for earliest divergence of quartzite-endemics (paired t = 5.6738, p < 0.01). The mean age for the earliest shift to calcrete-endemism across all groups did not differ significantly from the proposed timing of tectonic uplift at 3 to 5 Ma (t_{3Ma} = 1.4399, p = 0.2233; t_{5Ma} = -0.6965, p = 0.5245).

Within each group, the estimated age of the emergence of shale- and calcrete-endemics did not differ significantly (paired t = -0.5219, p = 0.6293). In addition, age estimates for earliest shale- and calcrete endemics did not differ significantly from age estimates of earliest seasonal-endemics (paired t_{shale} = 0.828, p = 0.435; paired $t_{calcrete}$ = 2.7026, p = 0.0639).

Shifts to alluvial- and lowsands-endemism were comparatively infrequent, earliest shifts across all groups being observed from 0.60 to 3.51 Ma (mean = 2.33 Ma), and from 1.56 to 12.16 Ma (mean = 4.58 Ma), respectively. In both cases, age estimates for their earliest divergence was significantly younger than the earliest quartzite-endemics (paired $t_{alluvial}$ = 3.2626, p < 0.05; paired $t_{lowsands}$ = 4.6853, p < 0.01).

3.3.4 Vegetation type

Reflecting the prevalence of fynbos endemics across all groups, the majority of the ancestral lineages were reconstructed as inhabiting fynbos vegetation (Figure 3.18). The only group that deviated substantially from this pattern was Arctotidinae, whose root node was reconstructed as polymorphic for fynbos and renosterveld (dated to 11.91 Ma). Within this group, two shifts into fynbos precipitated the emergence of small fynbos-dominated clades, the younger one being endemic to fynbos (8.92 and 5.68 Ma). Mean age estimates for the oldest fynbos endemics were significantly different only from the mean age estimates of succulent karoo endemics (Kruskal-Wallis $\chi^2 = 13.36$, $p < 0.01$), both when taking only the oldest shifts and all shifts into account.

3.4 Diversification rate analyses

Patterns of lineage accumulation across the eleven groups are depicted as log-lineages-through-time (LTT) plots in Figure 3.20. Visual inspection of the plots suggests that lineage accumulation across all groups proceeded more or less linearly over time, with the exception of *Pentameris*, *Elegia/Thamnochortus* and *Stoebe*. For these three, lineage accumulation appears to have been faster than in the other groups. This pattern is supported by diversification rate estimates calculated for each Cape clade, summarized in Table 3.5a (calculated using the method of Magallon & Sanderson 2001). In the absence of extinction ($\varepsilon = 0.0$), rate estimates for the 11 groups varied from 0.11 to 0.45 net speciation events per million years (Ma^{-1}). In contrast, assuming high relative extinction rates ($\varepsilon = 0.9$), lineage diversification rates were nearly halved (0.04 to 0.23 Ma^{-1}). Consistent with the patterns illustrated in LTT plots, the highest rate estimates under both low and high relative extinction rates were found in *Pentameris*, *Elegia/Thamnochortus* and *Stoebe*, while the lowest rate estimate was found in *Disperis*.

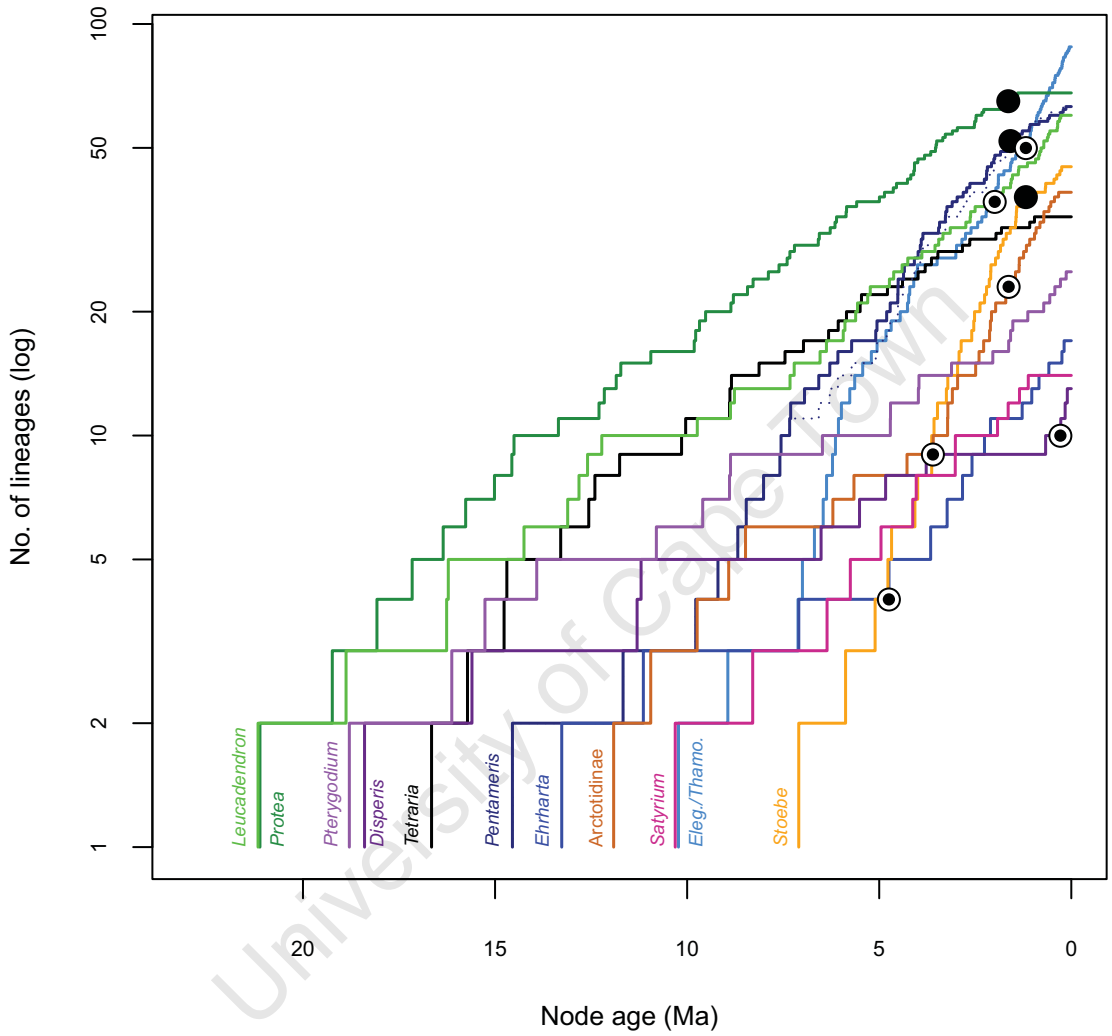

Figure 3.21 Log-lineage-through-time plots for the eleven groups depicting lineage accumulation of `CFR-centered' taxa within each sampled clade. Dashed line depicts the accumulation of lineages in te PentamerisNR clade, while the soild line of the same colour depicts lineage accumulation in the PentamerisCP dataset. Solid circles mark the point at which a significant decrease in a lineage's diversification rate occurred (here *Protea*, *Pentameris*, *Stoebe*). Solid circles within white circles mark the point in time when significant increases in diversification rates occurred (*Leucadendron*, *Pterygodium*, *Dipseris*, *Ehrharta*, Arctotidinae, *Elegia/Thamnochortus*). For more details on the results of diversification rate analyses, see Table 3.5.

Significant shifts in diversification rate were found in nine of the 11 groups (Table 3.5b), the timings of which ranged from 0.28 Ma to 4.75 Ma ago. With the exception of *Satyrium* and *Tetraria*, diversification in all groups was best described by a 'yule2rate' model of diversification (Table 3.5b). Under this model, rates are allowed to undergo a single rate switch either from slow to fast rates, and vice versa. Three of the groups, *Pentameris*, *Protea* and *Stoebe* showed decreases in diversification rate at 1.58, 1.64 and 1.42 Ma, respectively. In contrast, the remaining six groups underwent a rapid increase in their respective diversification rate, for which rate shifts occurred over a relatively wide time span (0.28 - 4.75 Ma). For *Satyrium* and *Tetraria*, the DDL model of linear density-dependent rates of lineage accumulation best described the data.

Table 3.5 Summary of diversification rate analyses using (a) the rate estimate (Magallon & Sanderson 2001) under zero and high relative extinction rates (ε) and the estimated number of taxa (N) missing from the data set, and (b) rate-variable diversification models (Rabosky 2006a) that were selected as the best fit to patterns of lineage accumulation for the 11 clades, with parameter estimates given for each model. For the yule2rate model, p_1 is the initial diversification rate, p_2 is the diversification rate subsequent to the switch, and t is the time at which the rate shift has occurred. For the DDL model, p_1 is the initial diversification rate and p_2 is the K or 'carrying capacity' parameter of the logistic density dependent model.

Group	a. Rate estimate			b. Rate variation model			
	ε = 0	ε = 0.9	N missing	model	p_1	p_2	t
Arctotidinae	0.259	0.1356	5	yule2rate	0.1534	0.3637	3.602
Stoebe	0.4451	0.2352	2	yule2rate	0.5813	0.1571	1.418
Disperis	0.1095	0.0447	2	yule2rate	0.0856	0.8856	0.284
Pterygodium	0.1441	0.0696	4	yule2rate	0.1129	0.2639	1.631
Satyrium	0.2017	0.0837	2	DDL	0.3595	20.1265	NA
Pentameris	0.2454	0.1394	0	yule2rate	0.2862	0.1093	1.584
Ehrharta	0.1773	0.0789	3	yule2rate	0.0776	0.2916	4.748
Elegia/Thamnochortus	0.3786	0.2249	2	yule2rate	0.3233	0.4801	1.182
Tetraria	0.1842	0.0958	9	DDL	0.2602	52.0874	NA
Protea	0.1683	0.0954	0	yule2rate	0.1630	0.0179	1.640
Leucadendron	0.1782	0.1044	25	yule2rate	0.1537	0.2498	1.993

3.5 Morphological divergence

Contrary to expectation (Figure 1.3), divergence events optimized to seasonal environments were not consistently associated with higher morphological divergence than those optimized to aseasonal environments. The relationship between seasonality and morphological differentiation was found to be significant only in three cases (Figure 3.21), and these did not consistently conform to the predicted pattern (Figure 1.3). Firstly, in *Tetraria*, spikelet length was found to decrease with increasing seasonality (ARST = 10.814, $p < 0.001$). Secondly, in sister-species-pairs analyses, plant height and leaf width were found to decrease and increase, respectively, with increasing seasonality (Plant height: ARST = 9.178, $p < 0.01$; Leaf width: ARST = 2.814, $p < 0.1$).

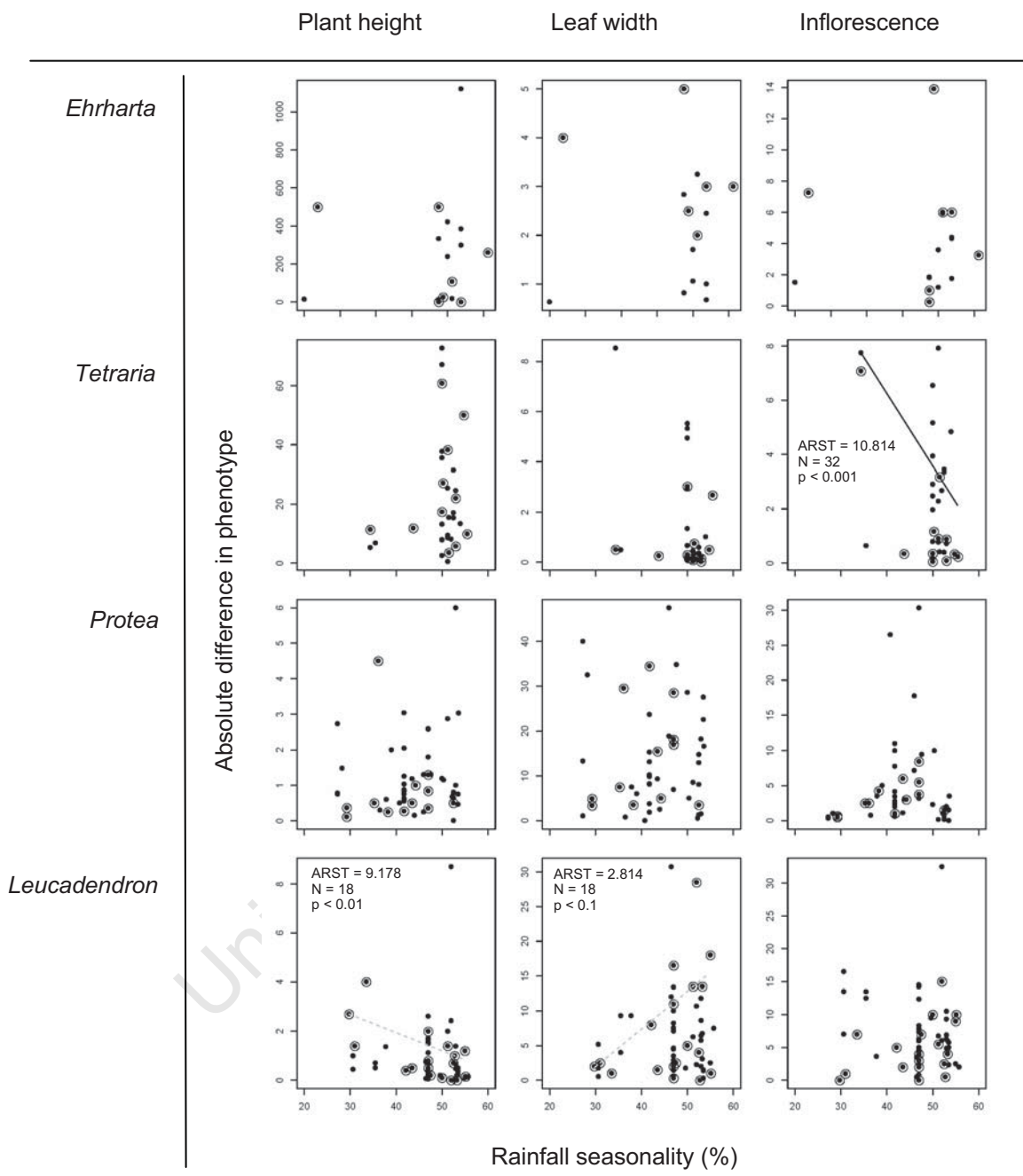

Figure 3.22 Morphological trait divergence across the seasonality gradient. Low values of seasonality depict aseasonal conditions, while higher values depict strongly seasonal conditions. Solid lines depict the 75% regression line across the entire phylogeny. Dashed lines depict the 75% regression line only across youngest tip nodes (direct sister species pairs). Asymptotic rank scores (ARST), the number of cases (N) and the p-value are provided where the observed pattern was statistically significant. `Inflorescence' describes different traits across the four groups; spikelet length in *Ehrharta* and *Tetraria,* tube length in *Protea*, and cone cross section in *Leucadendron*.

CHAPTER 4: DISCUSSION

Notwithstanding the analytical challenges involved, reconstructions of historical habitats for eleven lineages of Cape plants consistently identify an association with moist, aseasonal climates and quartzitic substrates as ancestral. The estimated dates on these ancestral nodes are variable, ranging from approximately 7 to 21 Ma, suggesting that this habitat has remained relatively stable since the mid-Miocene at least, and possibly acted as a refugium for early Cape floral elements. Consistent with palaeontological and environmental proxy data (Coetzee 1978a, b; Zachos *et al.* 2001; Dupont *et al.* 2011), the switches to dry, seasonal environments are consistently recent, commencing around 7 Ma, as indicated by the earliest divergence of seasonal-endemics in this study's Cape clades. Interestingly, the emergence of shale- and calcrete-endemics in the 11 clades is slightly delayed, commencing around 6 Ma in both cases. This suggests that occupation of these substrates by the modern flora did not occur as a simple function of climatically-induced extinction of their pre-existing floras. Although I tested the hypothesis that late Miocene-Pliocene environmental evolution precipitated floristic radiation, there was no consistent pattern in diversification rate shifts across the 11 clades. Similarly, I found no evidence to indicate a stronger role for adaptive divergence in seasonal than in aseasonal environments. Overall, the results of this study yield valuable insights into the historical evolution of the Cape environment, although it remains unclear exactly how environmental change has influenced floristic diversity.

It has long been believed, on the basis of palaeontological and environmental proxy data, that CFR climates deteriorated markedly through the Mid Miocene and early Pliocene (Coetzee 1978; Scott 1995; Zachos *et al.* 2001), and that this precipitated a significant reorganization of the vegetation (Levyns 1964; Linder *et al.* 1992). The timing of these climatic shifts is imprecise, however, owing to a paucity of evidence and poor resolution of the records used for palaeoenvironmental reconstruction (such as the fossil record; Scott 1982; Coetzee 1983). While fossil pollen analysis is a useful tool for reconstructing past vegetation types and climates, it lacks the spatial resolution that is needed to infer a detailed model of palaeofloristic evolution of the region (Scott 1982), and more specifically for the modern Cape flora. Nevertheless, fossil data have provided invaluable insights into the palaeofloristics of the region; for example, the pollen record reveals that, while many typical

elements of the Cape flora had a deep origin in the region (Coetzee 1978; Scholtz 1985; Linder 1987; Dupont *et al.* 2011), the modern Cape flora came to dominate the region only in the more recent past (~3 Ma; Coetzee 1978; Dupont *et al.* 2011). The data analysed and presented in this study considerably refine this picture. Results from ancestral state reconstructions using species' habitat profiles from 10 of the 11 study groups (excluding Arctotidinae) suggest an origin for the Cape flora in an oligotrophic, aseasonal (mesic) fynbos habitat. This is in stark contrast to the summer-arid Mediterranean climate that governs much of the region today, but, on a more local scale, reflects the physical conditions of the montane habitats in the CFR. Here, nutrient-poor, sandstone-derived soils dominate (see Figure 2.3) and the formation of 'south-easter' cloud belt at high altitudes likely ameliorates the impact of seasonal aridity in summer (Marloth 1904, 1908; Nagel 1962; Fuggle & Ashton 1979). Age estimates for the two oldest groups, *Leucadendron* and *Protea*, constrain the origin of this ancestral habitat to the Early Miocene (~21 Ma), and confirm an ancient origin for certain elements of a flora (e.g.: Restionaceae, Linder *et al.* 2005; Fabaceae, Edwards & Hawkins 2007; Bruniaceae, Quint & Classen-Bockhoff 2008) that came to dominate the region only in the past few million years (as revealed by fossil evidence, Coetzee 1978; Linder 2003; Dupont *et al.* 2011). Arguably more important, however, is that the range of age estimates for this ancestral habitat (7 – 21 Ma) suggests that it has persisted in the matrix of successive palaeoenvironments since the Early Miocene. This was possibly facilitated by relative climatic stability, as argued by Jansson and Dynesius (2002), which, in turn, allowed the persistence and gradual accumulation of species within moist 'refugial' habitats at higher altitudes, in particular during the most recent Plio-Pleistocene glacial period. This inferred role of these montane environments as climatic refugia in the CFR is further supported by the distribution of Cape flora palaeoendemics confined predominantly to higher altitudes (Goldblatt & Manning 2002; Warren & Hawkins 2008).

The value of these results is, of course, dependent on the accuracy and precision of the dating analyses presented. From this perspective, the existence of significant discrepancies between the age estimates reported here and those reported in earlier studies is of some concern. Specifically, my age estimates for the higher-level phylogenies of Orchidaceae, Asteraceae and Poales, as well as for the species-level phylogenies of *Stoebe*, *Ehrharta* and *Elegia/Thamnochortus*, are considerably younger than published age estimates while my age estimates for Arctotidinae are substantially older (see Table 3.2). These discrepancies could

reflect a variety of causes. In this study, the most likely explanations are differences in the dating methods employed (e.g.: methods which assume rate autocorrelation versus those that do not), the number and types of calibrations used (e.g.: fossil versus external rates calibration), and the phylogenetic placement of fossils (e.g.: stem versus crown node calibration). Comparisons between age estimates obtained in this study and published age estimates reveal that the choice of dating method is potentially highly influential in generating discrepant age estimates. Matching similar trends reported in earlier studies (Linder *et al.* 2005; Ho *et al.* 2005; Rutschmann 2006; Nowell 2008), I found that age estimates from methods which assume rate autocorrelation (PL, NPRS, and Multidivtime) were consistently older than those obtained here using a method which does not assume rate autocorrelation. By contrast, where ages were previously obtained using the same uncorrelated-rate model as employed in this study (i.e. BEAST), the dates reported here were either slightly younger or older than those reported previously, and the discrepancies were much smaller. The use of different calibrations may also have introduced discrepancies (e.g.: fossil versus external rate calibration). For example, where this study employed an indirect calibration obtained from a fossil-calibrated higher-level phylogeny (Asteraceae, this study) for dating Arctotidinae, McKenzie and Barker (2008) used an external substitution rate obtained from previous studies to calibrate branch lengths. Since rates are known to vary substantially between lineages (see Ho 2007) and their estimation is highly dependent on the method of analysis used to obtain these rates, the validity of using externally-determined rates is questionable (Hugall & Lee 2004). Finally, differences in age estimates may have arisen where fossil calibrations were variously referred to the crown versus the stem nodes of the clades which they represent (Renner 2005; Forest 2009). For example, the age estimates obtained for Orchidaceae in this study were consistently younger than those obtained from Gustafsson *et al.* (2010), despite the fact that both sets of dates were obtained using BEAST, near-identical sequence alignment and an identical set of calibration references. The key difference between the two studies, however, was that Gustafsson *et al.* (2010) placed fossil calibrations on crown instead of stem nodes. In a similar manner, discrepancies between the age estimates obtained in this study and those obtained by Bouchenak-Khelladi *et al.* (2010) for Poaceae are likely the result of the crown node of the BEP-PACCAD clade (BEP: Bambusoideae, Ehrhartoideae, and Pooideae; PACCAD: Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae, Aristidoideae, and Danthonioideae) having been calibrated to 57 Ma in the earlier study (based on a multi-

flowered grass spikelet fossil; Crepet & Feldman 1991). In this study, by contrast, this fossil was placed on the stem node of the BEP-PACCAD clade. In addition, I dated the crown node of this clade to 35 Ma, which defines the divergence of the BEP and PACCAD clades on the basis of phytolith data (Strömberg 2005). These phytolith data were, however, not used in the dating analysis by Bouchenak-Khelladi *et al.* (2010). In summary, in the majority of cases where age estimates differed substantially, discrepancies can be explained by differences in the methodology and calibrations adopted for dating analyses. The analyses presented here are based fundamentally on primary fossil calibrations conservatively attached to stem nodes, and do not assume rate autocorrelation, so the age estimates obtained are comparatively robust and likely constitute realistic estimates of divergence times.

Although the onset of pronounced seasonality in the Late Miocene has been identified as a critical stimulus for adaptive radiation in the Cape flora, both by Margaret Levyns and subsequent authors (Levyns 1964; Linder *et al.* 1992; Richardson *et al.* 2001; Verboom *et al.* 2004), the data presented here provide little evidence for a consistent rate shift to higher diversification rates over this period. Significant increases in diversification rates were found only in six of the 11 clades (Arctotidinae, *Disperis*, *Pterygodium*, *Elegia/Thamnochortus, Ehrharta* and *Leucadendron*), and only in two of these clades did the estimated timing in diversification rate change coincide with the inferred Late Miocene/Pliocene aridification (i.e. Arctotidinae at 3.60 Ma, and *Ehrharta* at 4.75 Ma). In accordance with the adaptive radiation model, the timing of this rate shift coincided with a habitat shift to non-quartzitic, seasonal, arid habitats in *Ehrharta* (also Verboom *et al.* 2004), but in Arctotidinae, it did not. In the remaining four 'rate-increasing' clades, the timing of diversification rate shifts occurred only much later, i.e. in the last 2 Ma, and did not consistently coincide with transitions to seasonally-arid environments. The five clades for which no significant increases in diversification rates were found showed either a significant decline in diversification rate around ~1.6 Ma (*Protea*, *Pentameris*, and *Stoebe*) or else showed diversity-dependent rate declines (*Tetraria* and *Satyrium*), possibly indicating 'ecological saturation' following radiation into the ecological niche space (Rabosky & Lovette 2008a; Slingsby 2011). In addition, divergence in morphological traits across four selected clades (*Tetraria*, *Ehrharta*, *Protea* and *Leucadendron*) provides little support for the hypothesis that Late Miocene climatic change proved conducive to adaptive radiation (see Figure 3.22). Enhanced phenotypic differentiation between lineages diverging in seasonal habitats was

found only in *Leucadendron*, for which morphological differentiation in leaf width between sister-species was greater in the novel adaptive zone, i.e. in the seasonal habitats. For the remaining comparisons, differentiation in selected phenotypic traits did not show patterns consistent with the hypothesized model (Figure 1.3).

In contrast to past climatic conditions, the geology and geomorphology of the Cape are generally assumed to have remained relatively stable through much of the Cenozoic (Hendey 1983; Tinker *et al.* 2008a, b). This is, however, challenged by landscape evolution models which suggest that the exposure of substrates (shales, granites, calcretes) associated with the incised valleys (Storms River, Great Fish, Kei, Sundays River) and much of the coastal plain of the CFR was a comparatively recent episode that entailed Early Pliocene tectonic uplift (3 -5 Ma) and coincided with an increase in erosion (King 1978; Partridge & Maud 1987). According to these authors increased erosion rates in the CFB region occurred in response to pulse(s) of Pliocene uplift along the Ciskei-Transkei flexure axis. For the first time, this exposed large expanses of shale (and granite) which underlie the quartzites of the Cape Supergroup. These finer-grained rocks have produced fertile, clay-rich soils drastically different to the acidic, nutrient-impoverished soils of the adjacent, largely quartzitic mountains (Lambrechts 1979; Deacon *et al.* 1992). As proposed by Cowling et al. (2009), the appearance of this matrix of younger landforms presented a 'novel' habitat for colonization and new opportunities for edaphically-driven radiation at that time. For the set of taxa studied here, the recent emergence of plant lineages endemic to these substrates is consistent with this hypothesis, with earliest shifts to shale substrates not differing significantly from the proposed episode of tectonic uplift at 3 – 5 Ma. Similarly, earliest shifts to calcrete substrates, formed on uplifted coastal platforms, did not differ significantly from the proposed timing of later Pliocene uplift (~5 Ma). Assuming, then, that the shale expanses of the lowlands and calcrete formations of the coastal margin had not been exposed prior to this uplift and, hence, that shifts to these substrates mark their earliest emergence in the region, these mostly Early-Pliocene transitions onto shale and calcrete substrates may then reflect an episode of regional tectonic uplift. Since both traditional and current geomorphological methods (e.g. anecdotal and cosmogenics/apative fission track analyses, respectively) lack the spatial and temporal resolution at this finer scale, these estimates, constrained by molecular clocks in biotic indicators, set the most accurate constraints of when- and possibly where- this Pliocene uplift event altered landforms and/or forged new

landforms in the Cape. Moreover, in four of the six clades showing increases in diversification rate, the timing of rate shifts coincided with the emergence of clades that diversified on shale and calcrete substrates (*Ehrharta*, *Thamnochortus*, and *Leucadendron*). This finding constitutes empirical support for the hypothesis of Cowling *et al.* (2009) for a primary role of tectonic uplift in triggering lineage divergence in Cape floral clades. In support of the Cracraft model (Cracraft 1985, 1992) for an ultimate control of tectonism over biotic diversification, the present study provides fine-scale data in support of Partridge *et al.*'s argument (1995) that Africa's uplift in the late Cenozoic was the ultimate driver of biotic evolution. While the latter paper argued for consequent climatic changes as the actual agent of biotic diversification, this study suggests that landform evolution was the dominant mechanism.

Nevertheless, the delayed occupation of shale lowlands may have also been precipitated by climatically-forced retreat of a pre-existing flora. The foregoing discussion notwithstanding, climatic aridification of the region in response to global cooling (Zachos et al. 2001), strengthening of the BUS (Siesser 1978) and possibly tectonic uplift (Linder 2003) could equally likely have precipitated the patterns of substrate shifts as described above. The modern Cape flora is generally thought to have spread across and become dominant in the CFR only in the past 3 – 4 Ma (as shown in the fossil record, Coetzee 1978), precipitated by aridification and climatically-induced retreat of the previous subtropical flora, possibly a woodland vegetation-type such as that described from Langebaanweg (Levyns 1964; Linder *et al.* 1992). In this light, it is the retreat of this flora, which allowed elements of the Cape flora to colonise the more fertile shale substrates that dominate the Cape lowlands. The formation of calcretes could also have occurred in response to the establishment of seasonal climates or due to fluctuating sealevels that were most severe during the more recent glacial period, the latter being reflected by the pulse of calcrete-endemics that emerge in the Pleistocene (see Figure 3.15). Hence, the comparative youthfulness of the modern shale- and calcrete-endemic flora revealed in this study could reflect the combined roles of both neotectonic uplift and climatic deterioration, but their specific impacts remain difficult to tease apart.

Whether a consequence of shale substrates being recently exposed, or their recent vacation by an earlier woodland flora, the youthfulness of the modern shale flora of the CFR may explain the general lack of shale-endemics detected in this study. Despite occupying at least

35% of the total landsurface of the CFR (Figure 2.4), only 5% of species in the 11 study groups were identified as shale-endemics. This is in stark contrast to the situation for quartzites, which are of similar spatial extent, but whose endemic flora comprises 51% of the surveyed species. Interestingly, about 7% of species were calcrete endemics despite the fact that exposure of calcretes is also recent (mid-Neogene) and calcretes are much more limited in extent (5% of total area).This suggests that the low numbers of shale endemics reported is not easily attributable to the recent exposure of the shale substrates and the youthfulness of their endemic flora alone. Alternative explanations include errors in the identification of substrate endemics, anthropogenic destruction of a once-much richer shale flora, and the existence of potential bias in the set of lineages sampled.

The identification of substrate-endemics was based on habitat profiles compiled from georeferenced herbarium records and digital lithology maps, an exercise that has been made possible with digitization of herbarium collection records. While this GIS-based classification approach is relatively objective and readily implemented, however, errors in substrate classification can easily be introduced due to poor resolution of the digital lithology layer or due to georeferencing inaccuracies (Kozak *et al.* 2008). This is particularly problematic for discrete variables such as substrate and vegetation type, since boundaries are digitally mapped as 'hard bounds'. Hence, even slight inaccuracies in the georeferenced locality point can result in incorrect substrate assignment. In the lowlands of the CFR, this is exacerbated by high edaphic heterogeneity which results in a high 'turn-over' of substrates across small spatial scales (Lambrechts 1979; Deacon *et al.* 1992; Figure 2.3). Nevertheless, this may be less important for shale endemics, since the shale substrates (Malmesbury shales in the West, Bokkeveld shales in the East) are generally exposed as large, continuous tracts of landscape. Furthermore, despite the potential pitfalls of the GIS approach, expert-opinion classification of substrate-occupation and endemism for *Stoebe, Pentameris, Ehrharta*, *Elegia/Thamnochortus*, *Leucadendron* and *Protea* exhibited comparatively minor disagreement with GIS-based substrate scoring. Moreover, where disagreements were substantial (i.e. *Ehrharta* and *Pentameris*), this was largely attributable to minor differences in polymorphic state assignments. To conclude, the under-representation of shale endemics is unlikely to be an artefact of the GIS-based methodology.

Alternatively, anthropogenic habitat transformation of the lowlands may have resulted in the extinction of the sampled clades' shale-specialists. Intensive agriculture in the Cape

dates back to the 17th century with the burgeoning colonization by European settlers (Deacon 1992 and references therein). To date, an approximate 25% of the region's total area has been transformed for agricultural purposes (Rouget *et al.* 2003; Raimondo *et al.* 2009), the lowland areas being most heavily impacted, because they embrace the more fertile shale-derived soils (Boucher & Moll 1981; Deacon *et al.* 1992; McDowell & Moll 1992; von Hase *et al.* 2003; Raimondo *et al.* 2009). The loss of lowland habitat was already noted in the early 20th century by Adamson (1938), with renoster shrubland currently being decimated to less than 10% of its original extent in the western and south-western coastal lowlands (Kemper *et al.* 2000). These anthropogenic impacts on the lowland flora underpin the argument that disproportionately more species have been lost in the lowlands compared to montane landscapes, most of which remain relatively pristine probably due to infertile soils and topography (Deacon *et al.* 1992; Richardson *et al.* 1995). The impact of past anthropogenic activity on the flora of the more fertile lowlands in the Cape is, however, difficult to assess. Nevertheless, given that an estimated 30 species are extinct from the entire South African flora (Raimondo *et al.* 2009), it is unlikely that extinction of shale-endemics in response to anthropogenic habitat degradation and loss would result in such discrepant representation of shale- versus quartzite endemics in a sample of Cape clades.

An alternative, and in my opinion a more plausible explanation for the scarcity of shale endemics is that sampling in this study was biased towards those lineages (e.g. members of Restionaceae, Proteaceae and schoenoid Cyperaceae) which show specialization to conditions of extreme nutrient deficiency and thus do not predominate on the more fertile soils of the CFR (Taylor 1978; Stock & Verboom 2012). These shale-derived soils are instead dominated by geophytes (Iridiceae, Orchidaceae, Oxalidaceae, Hyacinthaceae; see Proches *et al.* 2006), grasses, shrubs and herbs of Asteraceae, Malvaceae and Fabaceae (Goldblatt & Manning 2002; von Hase *et al.* 2003). Even though members of Asteraceae, Orchidaceae and Poaceae were sampled here, several of the particular clades sampled (*Stoebe*, Coryciinae, *Satyrium*, and *Pentameris*) are more closely affiliated with the oligotrophic than to the eutrophic environments (see Figure 3.18). Hence, the omission of true lowland-elements, such as members of the geophytic flora, which nevertheless represents 17% of the entire Cape flora (Goldblatt & Manning 2002), may explain the underrepresentation of shale endemics in this study. This, in turn, raises the question of what factors, by implication, limit floristic exchange between oligotrophic and eutrophic habitats.

In a large measure, the floristic distinction between plant assemblages on quartzite- versus shale-derived soils may be attributable to nutritional adaptations of different lineages which prevent large-scale exchange across the oligotrophic-eutrophic divide (Stock & Verboom 2012). While specialist adaptations for nutrient acquisition from the phosphorus-deficient soils of the CFR, such as cluster roots and mycorrhizal symbioses (Lamont 1983, 2003; Lambers *et al.* 2008) are advantageous in low-nutrient environments, there is some evidence that they may impose severe costs on the competitive ability of oligotrophic-adapted lineages in more eutrophic systems (Cowling *et al.* 1992), possibly due to P-sensitivity and -toxicity (see Poot & Lambers 2008; Hawkins *et al.* 2008). Probably as a consequence of both this and of species' tendency to retain their ancestral ecological traits ('niche conservatism'; see Wiens & Graham 2005; Crisp *et al.* 2009), fundamentally low-nutrient adapted lineages that dominate the oligotrophic, quartzitic soils in the mountains are unlikely to experience shifts to the eutrophic soils typical of the lowlands, as observed in this study (with the exception of *Ehrharta*). Edaphic control on floristic exchange between the oligotrophic and eutrophic habitats may also explain the comparatively high number of calcrete-endemics. Being generally of sandy origin, these calcareous soils are possibly nutritionally more similar to the quartzite-derived soils than the shale-derived soils, which would allow for higher rates of floristic exchange across calcareous-quartzite soils.

Edaphic controls on the exchange of lineages between montane and lowland habitats probably explains the existence of distinct vegetation types in each: while fynbos is generally associated with less fertile, quartzite-derived soils (oligotrophic), renosterveld generally occurs on the moderately fertile, shale-derived soils (eutrophic) that dominate the lowlands (Taylor 1978; Cowling 1983). Despite incorporation of these vegetation types in a common Fynbos biome (*sensu* Mucina & Rutherford 2006), earlier accounts of the region's vegetation types describe the renosterveld as 'Cape transitional small-leaved shrubland' (Moll *et al.* 1984), as non-fynbos renoster-shrubland with a close affinity to karooid vegetation (Campbell *et al.* 1992) and as 'False karoo-type' (Acocks 1953). From these treatments, it is apparent that floristic overlap between the renosterveld and fynbos vegetation types is minimal. In this light, it is interesting how a consideration of more fertile-substrate lineages would affect our general picture of the timing in the appearance of endemism to different substrates in the CFR. Candidate groups include Aizoaceae (Klak *et al.* 2004), Iridaceae (*Babiana*, Goldblatt & Manning 2007a, b; *Moraea*, Goldblatt *et al.* 2002), Brassicaceae

(*Heliophila*; Mummenhoff *et al.* 2005), Oxalidaceae (*Oxalis*; Oberlander *et al.* 2011), Zygophyllaceae (*Zygophyllum*; Bellstedt *et al.* 2008a, b, pers. comm.) and Geraniaceae (*Pelargonium*; Bakker *et al.* 2005), which associate predominantly with the more fertile renosterveld habitats of CFR lowlands. The clades for which molecular date estimates are available reveal both recent diversification in lowland contexts (*Heliophila*: 2 - 5 Ma, Mummenhoff *et al.* 2005; Aizoaceae: 3.8 - 8.7 Ma, Klak *et al.* 2004) corroborating the recentness of the renosterveld/succulent karoo radiation in the CFR (Verboom *et al.* 2009). Others show recent origins in the CFR with relatively constant diversification rates (*Babiana*: ~7.5 Ma, Schnitzler et al. 2011), as well as groups having older origins (> 15 Ma) with relatively constant diversification rates throughout the Miocene (*Pelargonium,* Bakker et al. 2005; *Moraea*, Schnitzler *et al.* 2011) that diversified across a variety of habitats. Taking into consideration that some of these groups were dated using a variety of methods (PL, NPRS, Multidivtime, BEAST) which often generate discrepant age estimates (as shown in this study), a valuable exercise would be to investigate the diversification history of these groups within the same analytical framework as the 11 groups presented in this study. Hence, given that these groups likely represent more the lowland flora, their inclusion will broaden representation of the overall floristic diversity, and allow for a more extensive view on floristic evolution in the CFR to be inferred.

CONCLUSION

The combination of multiple molecularly-dated phylogenies and reconstructed species' ecologies, referred to here as the 'geoecodynamic' approach (Cotterill & de Wit 2011), provides novel insights into the history and palaeoenvironmental evolution of the CFR. Confidence in palaeoenvironment reconstruction is strengthened by the composite signal of phylogenetic congruence, testifying how independently evolving clades have occupied novel environments on comparable time scales, which allows reconstructions at a scale unattainable by the region's scarce fossil record. This study provides strong evidence that the oligotrophic, aseasonal (and relatively mesic) habitats typical of the higher altitudes likely represent the place of origin for many of the Cape clades, and possibly represent a set of conditions which were more prevalent prior to the Miocene-Pliocene boundary. Acting as isolated climatic refugia, these montane habitats allowed the persistence of lineages on the one hand, and gradual accumulation of species as range sizes expanded and contracted with climatic fluctuations and aridification, on the other. Hence, the diversity in the ancestral refugial habitats is likely to have been shaped largely by non-adaptive processes driven by climatically-forced fragmentation, producing lineages phenotypically and ecologically indistinct (see Britton 2009). Evolutionary processes that acted in the novel seasonal, eutrophic environments shaped by aridification and uplift are more difficult to infer due to biased lineage sampling. Limited transitions from the ancestral oligotrophic to the more recent eutrophic habitats by fynbos oligotrophic-adapted lineages probably reflect constrained floristic exchange between oligotrophic-eutrophic plant assemblages mediated by edaphic controls (Stock & Verboom 2012). This limited floristic exchange between plant assemblages on oligotrophic versus eutrophic soils likely manifests itself as floristic distinction between the region's dominant vegetation types, the fynbos and renosterveld. In this light, the incorporation of additional lineages, specifically those that associate with eutrophic soils (e.g.: Iridaceae, Oxalidaceae, Fabaceae, Aizoaceae, Brassicaeae, Zygophyllaceae), with the analytical framework presented here is a necessary step towards understanding the full evolutionary history of the CFR. This, then, is likely to provide insight into the predicted contrasting evolutionary histories of the lowland- (or renosterveld) and upland-flora (fynbos). In addition, even though the morphological divergence analyses presented here did not reveal consistent patterns as predicted, there is considerable scope for refining the methodology.

While analytical techniques and data sets pertinent to the geoecodynamic approach (i.e. molecular dating, ancestral state reconstruction, environmental GIS layers, and point locality data) are exceptionally powerful, they require careful implementation. As illustrated in this study, of utmost importance is the standardization of the dating techniques employed, as well as careful assessment of fossil selection and placement for branch length calibration.

Finally, syntheses of these data have enabled us to evaluate how well the genomic record of biotic indicators performs in reconstructing the spatial and temporal details of environmental events that changed Cape landscapes at the mesoscale. Molecular dating of these biotic indicators demonstrates the novel degree of spatial fidelity obtained, compared to traditional geomorphological methods. This progress obtained for the Cape and its biodiversity lends additional confirmation to a geobiological approach to quantify aspects of the geomorphic history of a landscape (Craw et al. 2008; White *et al.* 2009; Goodier *et al.* 2011; Schwarzer *et al.* 2011).

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APPENDIX

Table A.1. List of additional taxa used in higher-level dating analyses, Genbank accession numbers, and specimen vouchers.

Family	Taxon	Gene		Specimen Voucher
		ndhF	rbcL	
Cyperaceae	*Tetraria flexuosa*	-	unpublished	V505 Slingsby 2011
	Tetraria involucrata	-	AM234973	Balele K. 33 (NBG)
	Tricostularia pauciflora	-	AY725954	
Restionaceae	*Thamnochortus levynsiae*	-	AY690759	HP Linder, CR Hardy, Moline P 7345
	Thamnochortus sporadicus	-	AY690780	HP Linder, CR Hardy, Moline P 7341
	Thamnochortus glaber	-	AY690756	Cowling s.n.
	Rhodocoma fruticosa	-	AY690749	HP Linder, CR Hardy, Moline P 7609
	Rhodocoma capensis	-	AY690748	HP Linder, CR Hardy, Moline P 7248
	Askidiosperma paniculatum	-	AY881410	HP Linder, CR Hardy, Moline P 7378
	Chondropetalum nudum	-	AY881421	HP Linder, CR Hardy, Moline P 7219
	Staberoha multispicula	-	AY881473	HP Linder, CR Hardy, Moline P 7318
Poaceae	*Pentameris aurea*	EU400811	EU400671	Galley, C. 47 (Z)
	Pentameris airoides	EU400810	EU400670	Galley. C. 81 (Z)
	Tribolium brachystachyum	EU400823	EU400677	Verboom, G.A. 593 (BOL)
	Pseudopentameris macrantha	EU400816	DQ887122	Linder 5470 (BOL)
		matK	rbcL	
Orchidaceae	*Corycium orobanchoides*	EU301528	-	Pauw A 47 (BOL)
	Corycium microglossum	EU301526	-	Pauw A 16 (BOL)
	Corycium carnosum	EU301524	AY381115	Pauw A 30 (BOL)/ Chase M O-692 (K)
	Pterygodium catholicum	EU301533	AY368346	Pauw A DNA,28/ Chase MW O-1130
	Pterygodium caffrum	EU301525	-	Pauw A 27 (BOL)
	Ceratandra grandiflora	EU687535	-	Pauw & Liltveld 49 (BOL)
	Ceratandra bicolor	EU301541	-	Pauw A 2 (BOL)
	Evotella rubiginosa	EU301508	-	Pauw A 4 (BOL)
	Disperis capensis	AJ310022	AY381120	1203MWC/ Chase MW O-1203 (K)
	Disperis lindleyana	AY370652	AY370651	Chase MW O-696
	Earina autumnalis	AF263656	AF074155	
	Earina valida	AY121741	AF518051	C296
	Agrostophyllum majus	AY368391	AF518054	Chase MW O-562/ MWC1402
	Bulbophyllum lobbii	AY368395	AF074115	Chase MW O-474
	Dendrobium lindleyi	GQ248117	GQ248589	USBG 99-2351
	Dendrobium nobile	FJ216672	FJ216583	SMJC-SH
	Satyrium bracteatum	EF612540	-	BB2110 (BR, K, NBG, NY)
	Satyrium rhynchatum	EF612588	-	BB2155 (BR, NBG, Z)
	Satyrium acuminatum	EF612534	-	T18b (Z)
	Satyrium chlorochorys	EF612547	-	HK1969 (MAL, PRE,SRGH, UZL)
	Satyrium trinerve	EF612595	-	BB2255 (BR, NBG)
	Satyrium cristatum	EF612552	-	BB2297 (GRA, NBG)
	Satyrium nepalense	EF612575	-	Chase O-539 (K)

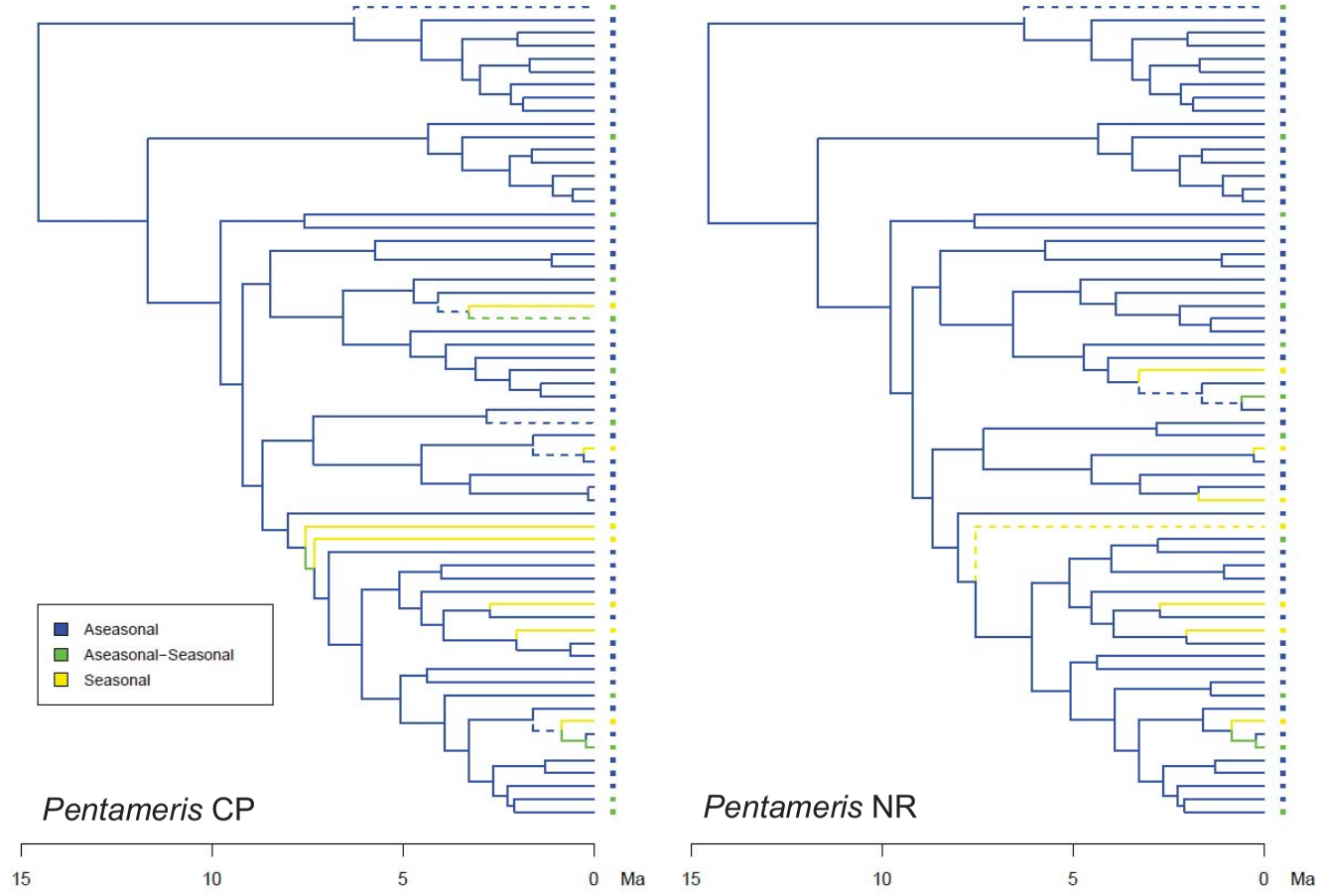

Figure A.1. Optimization of rainfall seasonality across *Pentameris* for the dataset comprising the 'chloroplast' (CP) accessions and 'nuclear' (NR). Filled boxes at the tips of the phylogenies depict the state of the extant taxa.

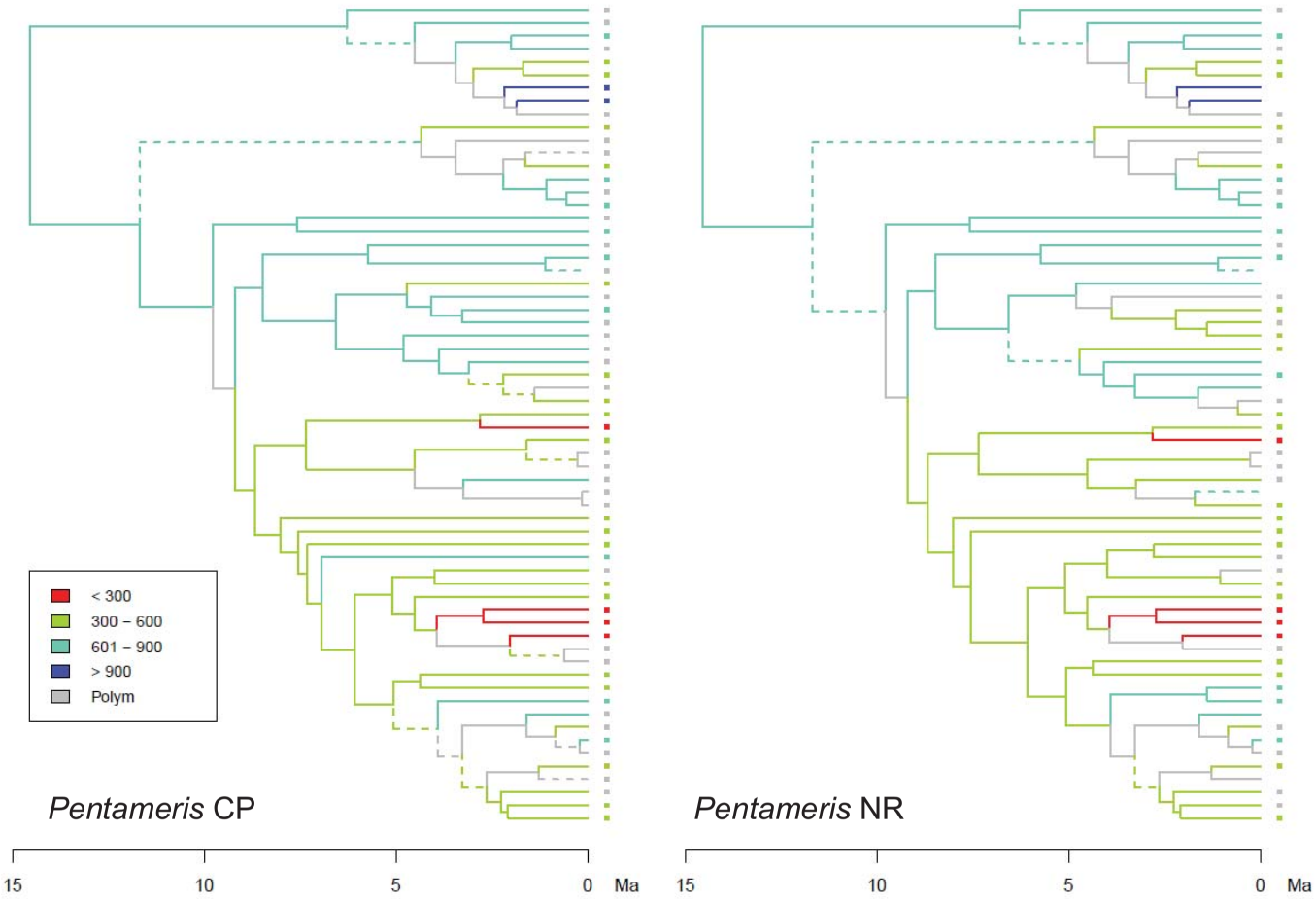

Figure A.2. Optimization of mean annual rainfall (mm) across *Pentameris* for the dataset comprising the 'chloroplast' (CP) accessions and 'nuclear' (NR). Filled boxes at the tips of the phylogenies depict the state of the extant taxa.

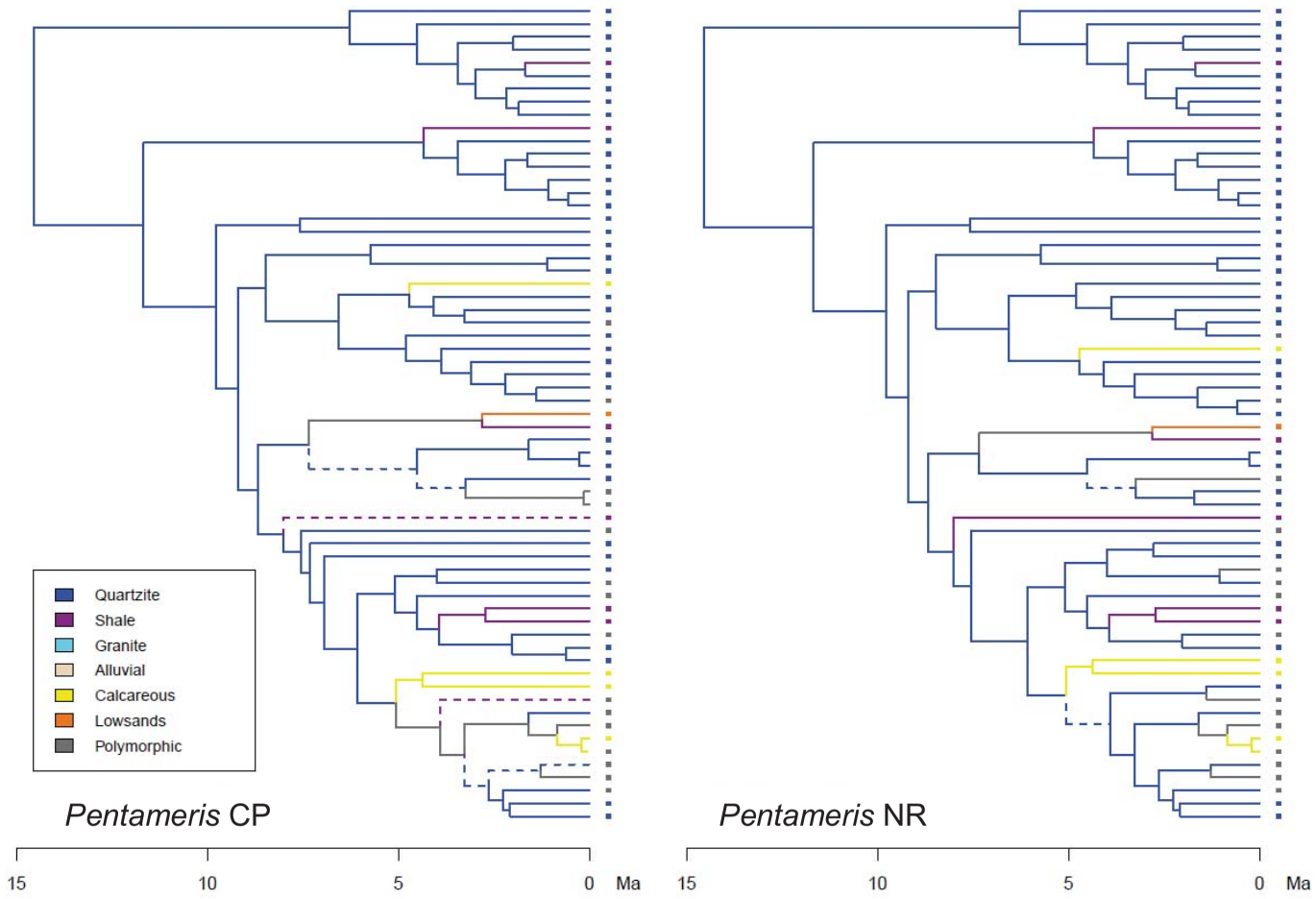

Figure A.3. Optimization of substrate type across *Pentameris* for the dataset comprising the 'chloroplast' (CP) accessions and 'nuclear' (NR). Filled boxes at the tips of the phylogenies depict the state of the extant taxa.

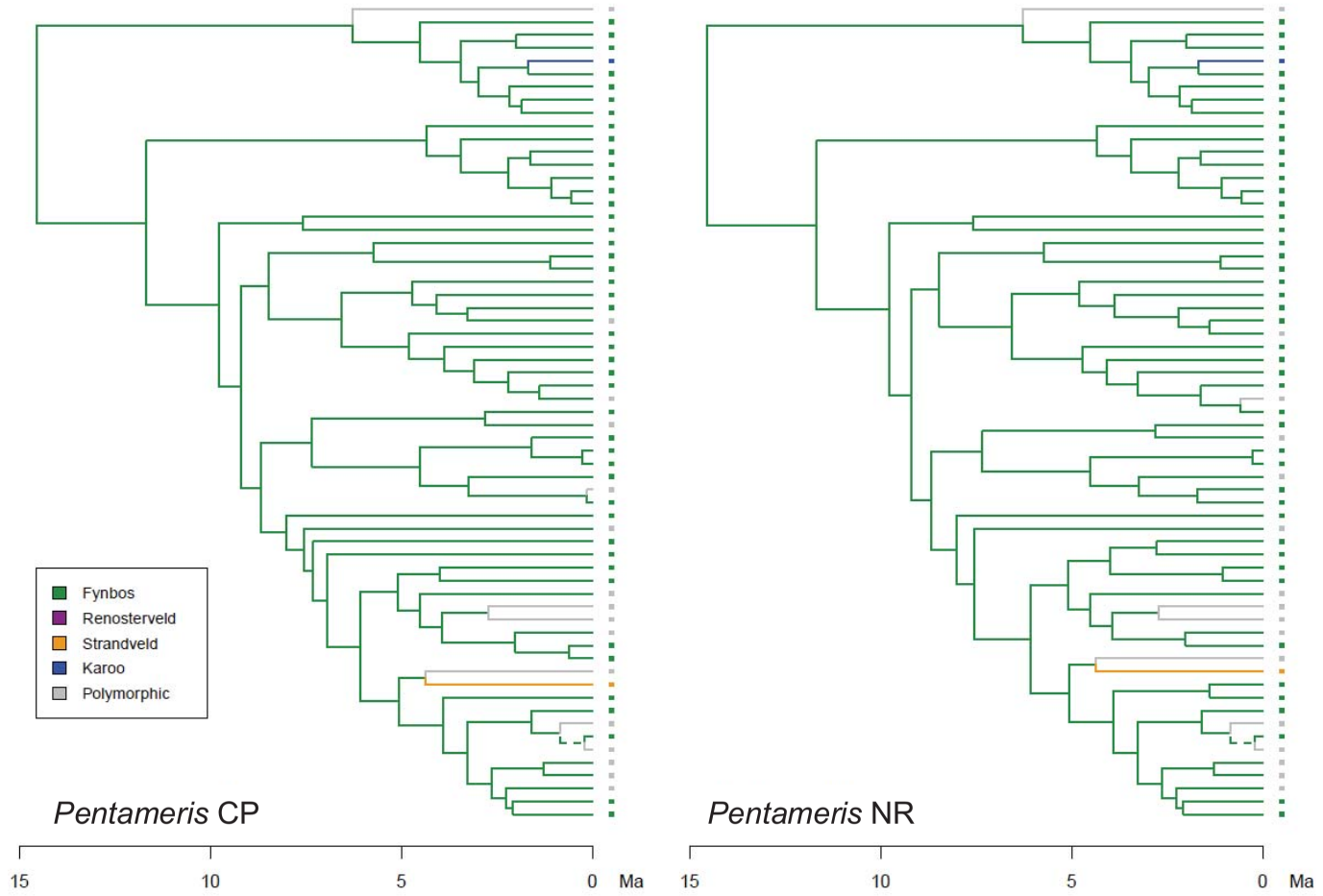

Figure A.4. Optimization of vegetation type across *Pentameris* for the dataset comprising the 'chloroplast' (CP) accessions and 'nuclear' (NR). Filled boxes at the tips of the phylogenies depict the state of the extant taxa.